THE RELATIONSHIP BETWEEN *Aeschynomene americana* L. AND ROOT-KNOT NEMATODES (*Meloidogyne* spp.)

By

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	iii
ABSTRACT	ix
CHAPTER I. INTRODUCTION	1
CHAPTER II. REVIEW OF THE LITERATURE	4
Aeschynomene	4
Root-Knot Nematodes (Meloidogyne spp.)	14
Root-Knot Nematode Control by Resistant Cultivars	21
CHAPTER III. NORTH CAROLINA HOST DIFFERENTIAL TEST	30
Introduction	30
Methods and Materials	31
Results and Discussion	34
CHAPTER IV. PARENT EVALUATION AND SELECTION	40
Introduction	40
Methods and Materials	41
Statistical Analysis	42
Results and Discussion	49
CHAPTER V. ARTIFICIAL HYBRIDIZATION OF Aeschynomene americana L	66
Introduction	66
Flowering Sequence	67
Artificial Hybridization	69
Precautions	74
Plant maintenance and Seed Collection	77

Page

CHAPTER VI. EFFECTS OF Aeschynomene americana ON THE LIFE CYCLE OF A ROOT-KNOT NEMATODE (Meloidogyne) SPECIES..... 79

Introduction..... 79
Methods and Materials..... 81
Results and Discussion..... 85

CHAPTER VII. THE INHERITANCE OF ROOT-KNOT NEMATODE RESISTANCE IN Aeschynomene americana L. 91

Introduction..... 91
Methods and Materials..... 92
Results..... 94
Discussion..... 107

APPENDIX..... 111

BIBLIOGRAPHY..... 115

BIOGRAPHICAL SKETCH..... 124

LIST OF TABLES

Table		Page
3-1.	Observed and Expected Reactions from the North Carolina Host Differential Test.....	37
3-2.	Gall and Egg Scores, and Perineal Patterns of the Plants Infested with the University of Florida Meloidogyne incognita Population.....	38
4-1.	Analysis of Variance for Gall and Egg Scores for the Complete Model.....	44
4-2.	Analysis of Variance for Gall and Egg Mass Scores for M. arenaria.....	45
4-3.	Analysis of Variance for Gall and Egg Mass Scores for M. javanica.....	45
4-4.	Analysis of Variance for Gall and Egg Mass Scores for M. incognita race 3.....	46
4-5.	Variances and Broad Sense Heritabilities for Gall and Egg Mass Scores Over All Species and for Individual Nematode Species.....	47
4-6.	Mean Gall Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne arenaria.....	51
4-7.	Mean Egg Mass Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne arenaria.....	52
4-8.	Mean Gall Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne javanica.....	53
4-9.	Mean Egg Mass Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne javanica.....	54
4-10.	Mean Gall Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne incognita race 3.....	55
4-11.	Mean Egg Mass Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne incognita race 3.....	56

Table		Page
4-12.	Analysis of Variance of Gall Scores for Experiment 2 with Both the SAS and Harvey Analyses	59
4-13.	Analysis of Variance of Egg Mass Scores for Experiment 2 with Both the SAS and Harvey Analyses	59
4-14.	Means and Variances of Gall and Egg Mass Scores of Seven Aeschynomene americana Lines Over Two Replications for M. arenaria and M. javanica	62
4-15.	Means and Variances of Gall and Egg Mass Scores of Seven Aeschynomene americana Lines Over Two Replications for M. incognita race 1 and 3	62
5-1.	Effect of Eleven Female Parents on Pod and Seed Production of Artificially Hybridized Aeschynomene americana L.	75
5-2.	Effect of Eight Male Parents on Pod and Seed Production of Artificially Hybridized Aeschynomene americana L.	76
6-1.	Nematode Life Cycle Stage Found in Three Aeschynomene americana Genotypes under Short days and Cool Temperatures, when Infested with M. incognita race 3.(Experiment 1)	86
6-2.	Nematode Life Cycle Stage Found in Three Aeschynomene americana Genotypes under Long Days and Warm Temperatures, when Infested with M. incognita race 3. (Experiment 2)	87
7-1.	Identification of Parents and F1's Used for the Evaluation of the Inheritance of Resistance to Root-Knot Nematodes	93
7-2.	Mean Gall and Egg Mass Score for All Generations and Families Inoculated with M. javanica	97
7-3.	Mean Gall and Egg Mass Score for All Generations and Families Inoculated with M. arenaria race 1	98
7-4.	Mean Gall and Egg Mass Score for All Generations and Families Inoculated with M. incognita race 3	99

Table		Page
7-5.	F2 Segregation Ratios for All Crosses Inoculated with M. javanica	100
7-6.	F2 Segregation Ratios for All Crosses Inoculated with M. arenaria race 1	101
7-7.	F2 Segregation Ratios for All Crosses Inoculated with M. incognita race 3	102
7-8.	F2 Segregation Ratios for All Crosses for All Nematode Species	106
A-1.	Data Summary from 1986 Experiment of Aeschynomene americana parents, F1's, F2's and Backcrosses which were Inoculated with Meloidogyne incognita race 3	112
A-2.	Data Summary from 1986 Experiment of Aeschynomene americana parents, F1's, F2's and Backcrosses which were Inoculated with Meloidogyne javanica	113
A-3.	Data Summary from 1986 Experiment of Aeschynomene americana parents, F1's, F2's and Backcrosses which were Inoculated with Meloidogyne arenaria race 1	114

LIST OF FIGURES

Figure		Page
2-1.	*Aeschynomene americana* Flower and Fruit.....	9
3-1.	Diagrams of Posterior Cuticular Patterns of *Meloidogyne* spp.....	35
5-1.	*A. americana* Half-Flower.....	70

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THE RELATIONSHIP BETWEEN Aeschynomene americana L. AND ROOT-KNOT NEMATODES (Meloidogyne spp.)

by

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Aeschynomene americana L. is an annual tropical legume used for its nutritional value as a forage for beef cattle. An improvement program was initiated in Florida to develop desirable agronomic traits and expand its commercial uses.

To precisely transfer traits between genotypes an artificial hybridization technique was developed. The method involved the removal of the stamens followed by controlled pollination. The risks of self pollination or contamination were minimized and the success rate was maximized. The success rate was not affected by the female or male parent used.

Root-knot nematodes (Meloidogyne spp.) are endemic to the tropics and hamper the use of susceptible forages.

A population of A. americana genotypes was individually examined for their responses to M. incognita race 1 and 3; M. javanica; and M. arenaria race 1. The population was found to contain a wide range of responses from resistant to susceptible.

Three plant genotypes with different responses were used to examine the effects of the genotype on the life cycle of M. incognita race 3 under two different day length and temperature regimes. The plants were examined weekly and the different nematode life cycle stages counted after staining. Development to maturity was reduced in the more resistant genotypes in both environmental regimes. Under long days and higher temperatures, surviving females in resistant genotypes could reproduce as well as those in the susceptible genotypes.

Two resistant and two susceptible plant genotypes which had uniform nematode responses were selected for the examination of the genetic system controlling the responses to three root-knot nematode species. They were crossed and their first filial generation (F1's) used to create F2's and backcrosses. All the generations were tested with each nematode species. The data from all the families and generations for the three nematode species were combined; there appeared to be one dominant gene for resistance common to all three species. There was evidence for other genes being involved which seemed to be partially dominant, and probably specific to the individual nematode species.

CHAPTER I
INTRODUCTION

Grassland research in Florida has concentrated on finding and developing suitable forage crops to supply the ever increasing cattle production in the state. Aeschynomene americana L. (American Joint Vetch) is an annual legume which is well adapted to the tropics and has been found to be a high value forage for cattle grazing in tropical and subtropical regions. However, A. americana does not produce a sustained production of forage material, and thus its use is limited to certain areas and times of the year. Therefore, in order to develop the desirable traits and expand the use of Aeschynomene as a grazing forage legume, a breeding and improvement program has been initiated.

Over 50 Meloidogyne species are distributed worldwide and can collectively attack every crop grown. The average crop losses due to these nematodes is estimated at 5% yearly worldwide, but can be as high as 60% in individual field situations (Taylor and Sasser, 1978; Carter and Sasser, 1982). Root-knot nematodes (Meloidogyne spp.) are endemic in Florida and responsible for yield losses in just about every crop grown in the state. It has been estimated

that M. arenaria was responsible for a loss of over $1.6 million per annum in peanut (Arachis hypogaea L.) production in Florida (Dickson, 1973). Root-knot nematodes are a recognized limiting factor in the establishment and growth of forage legumes. They may hamper the use of Aeschynomene in Florida and tropical areas where these nematodes are widespread and a serious problem. Environmental and economic constraints prevent the use of chemical control of nematodes, and thus the best alternative would be to develop resistant cultivars. A root-knot nematode resistant cultivar of A. americana would provide the farmer with a high nutritional value forage legume which would also be adapted to his subtropical conditions, as well as a means of reducing the root-knot nematode populations.

Improving a hitherto undomesticated species requires general background research; i.e. botanical characterization, collecting and screening large populations of germplasm etc. before starting the amalgamation of traits into an improved cultivar. Botanical information pertaining to the reproduction of the species enables one to select the most appropriate and feasible manner to physically alter or improve the species.

The traits have to be identified and genetically understood in order to amass them into one cultivar. This can not be achieved immediately, as some traits will be

controlled by complex genetic systems and others by simple systems. In order to facilitate the manipulation of a genetic system and do so in a precise, controlled manner, its gene action must be determined. Breeding for nematode resistance is complicated by the presence of two genetic organisms, which interact with each other as well as independently with the environment. Thus, to understand the gene action of the host, its relationship with the pest should also be examined.

The objectives of this investigation were (1) to determine the best method of artificially hybridizing A. americana; (2) to screen the collection of Aeschynomene germplasm for the range in responses to several root-knot nematode species and to select the parents which respond the most uniformly for further study; (3) to examine the effect of the host plant on the nematode life cycle in order to understand the relationship between the two organisms and the mechanism of resistance; and (4) to determine the mode of gene action of the response of A. americana to several root-knot nematode species.

CHAPTER II
REVIEW OF THE LITERATURE

Aeschynomene

Taxonomy

Aeschynomene is a genus of the legume family, characterized by papilionaceous flowers and jointed fruits or loments. The species in the genera range from herbaceous annuals to woody perennials. There are some 350 species; of which approximately half are of New World origin and half Old World (Africa). The plants may be erect to prostrate, but never vines. The leaflets are sensitive to light and often touch, and therefore are included in the category of 'sensitive' plants. The name Aeschynomene is Greek, meaning bashful or ashamed. The term "Aeschynomene" was used as a generic term for all the sensitive plants which led to the Pre-Linnaean writers classifying *Mimosa*, *Acacia*, *Cassia*, and the modern *Aeschynomene* together. Linnaeous validated the generic name *Aeschynomene* for modern nomenclature by including it in his first edition of "Species Plantarum" (1753). Of the 5 species he listed only 3 remain in the genus today. Over the next hundred years some 200 species were added. Since about 1850, the genus has been added to occasionally, mainly from collections in Mexico and Brazil (Rudd, 1968).

There has been some confusion as to the classification of the various species and varieties of Aeschynomene, mainly due to the presence or absence of glaberous hairs on various parts which were not always included in the descriptions. Rudd (1968) determined that Aeschynomene americana L. included three varieties, based on their anatomical similarities: americana, glandulosa, and depila. She also found that the species called A. mexicana and A. tricholoma were the same as A. americana. Common names vary with locations but include Aeschynomene, American joint vetch, Antejuela (El Salvador), Pega pega (Panama, Puerto Rico), Ronte (Haiti), Honteuse femelle (Martinique), Cujica (Venezuela), and Lajoumi (India = "sleep" movements).

Distribution

Aeschynomene is chiefly a tropical genus with a few species occurring in the warmer temperate climates. The overall latitudes range from 40°N to 35°S on the Atlantic coast and 28°N to 17°S on the Pacific coast, principally in the Americas, but it is also found in Africa, Southeast Asia, the Pacific islands and Australia (Paul, 1951; Rudd, 1968; Amaratunga, 1977; Dillon, 1980; Wilson, 1980). Most of the species are hydrophytes, generally found in wet areas, such as wet meadows, marshes and rice paddies, where they are a common weed problem. A few are xeric, occurring in savannas, rocky hillsides and

woodlands (Rudd, 1968; Allen and Allen, 1981). There seems to be no correlation between the species and habitats, indicating somewhat flexible edaptic requirements.

The origin, suggested by the great diversity of taxa found, is in the southern Sierra Madre region of Mexico (Rudd, 1968). Today, Aeschynomene seems most abundant in the Caribbean where its migration may have been facilitated by hurricanes. Hurricanes may well account for the distribution of Aeschynomene to the Florida peninsula where it was first recorded in Orange and Osceola counties in 1930, and Polk county in 1932 (Rudd, 1968).

Aeschynomene has been known in Java since 1855 when it was introduced and tested by planters for uses in tea, rubber and coffee estates, and since then it has travelled throughout the Indo-Malasian region. Presently it is regarded as a weed, green manure or soil cover crop in Ceylon, Indonesia and Malaysia (Paul, 1951; Ghosh and Dutta, 1976; Amaratunga, 1977). It has been suggested that the seeds were distributed as feed grain, ballast, air cargo or in packing material carried about during World War II, when military camps were built in the Pacific islands and India. It was found in India in the Ranchi district and in Sri Lanka growing spontaneously under rice paddies, and various wet places (Maheshwari and Ghosh, 1971). A. americana was introduced into Australia during the late 1960s as material for a new forage legume species (Wilson et al., 1982; Bishop et al., 1985).

Botanical Description

A. americana is classed as a herb. The plants are usually erect, but may be weakly so, generally ranging between 0.25 and 2 m tall. The roots bear nodules, characteristic of the Leguminosae, nodulating best with Rhizobium of Group I (Albrecht et al., 1981, Bishop et al., 1985; Quesenberry, 1981) and the nodules are well developed. The plants and associated bacteria show N2 fixation even when subjected to water-logged conditions for long periods of time (Albrecht et al., 1981; Miller and Williams, 1981; Bishop et al., 1985).

The plants are covered with varying amounts of glandular hairs, and may or may not have verrucose excrescences. The leaves, which are pinnately compound, are small and, reminiscent of the Mimosoideae, are somewhat sensitive to touch and light. The inflorescence is axillary, basically racemose and few flowered. The paired bracteoles immediately subtend the calyx, and completely cover the calyx in bud. The flowers are papillonaceous (5-membranous), zygomorphic, somewhat persistent and approximately 10 mm long when fully expanded.

The petals are of similar lengths but may vary in colorations. The keel is lightly fused along half its length. The stamens are diadelphous (5-5); the filaments are free from about midlength to the apex, below which they are united to form a sheath. At anthesis this sheath splits longitudinally to form two phalonges of five stamens

each. The ovary is 1 to 9 ovulate and superior. The style is flexible, curling back at the apex, and the stigmatic surface is minutely capitate (Rudd, 1968; Dillon, 1980). (Figure 2-1.)

Uses

World wide, Aeschynomene has been usually considered a weed, especially in rice paddies, but over the past 30 years cultivated uses, primarily as a forage, have been found and are being developed.

In the 1950s, A. americana found thriving on waste lands in Sri Lanka was found to be nutritionally comparable to alfalfa as a hay crop. At the same time it was used in Indonesia as a green manure and soil cover crop. Recently it was found to increase the yield and quality of rice and wheat when used as green manure in India (Maheshwari and Ghosh, 1971; Singh, 1971).

In 1985, the cultivar 'Glenn' of A. americana was released in Australia as a new forage legume, but its commercial use is as yet not widespread (Bishop et al., 1985).

By far the most work done with A. americana has been in Florida since the 1950s, when it was first found to be a possible forage legume (Kretschmer, 1967). Moore and Hilmon (1969) found that the young stems and leaflets were readily consumed by deer and cattle. A common ecotype of A. americana is currently available in Florida and is known

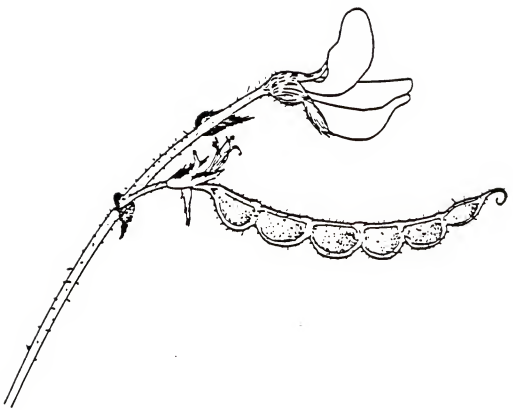

Figure 2-1. Aeschynomene americana Flower and Fruit. (x2.5)

by the common name aeschynomene. It was amassed from collections made throughout the state in the 1950s and described by Hodges et al. (1982). The use of this ecotype has been limited to the southeastern part of the U.S., primarily in Florida. Aeschynomene is tolerant of flooding and thus is well adapted to the poorly drained soils and seasonally flooded conditions of parts of Florida (Albrecht et al., 1981; Miller and Williams, 1981).

Production Practices

A. americana exhibits a short-day floral initiation habit (Kretschmer and Bullock, 1980) and flowering of the Florida common ecotype occurs about the 10 Sept. in North Central Florida (Quesenberry and Ocumpaugh, 1981). It produces large quantities of seed which generally remain in the pod, whether they drop from the plant or are combined (Ruelke et al., 1974; Hodges et al., 1982). Pod removal and mechanical scarification increases germination from 5% to 78% (Hanna, 1973) and to 98% (Ruelke et at., 1975; Tang and Ruelke, 1977). Seed with or without the pod can be obtained commercially and both types may be useful, depending on the conditions at planting. If moisture is available, naked seed is desirable due to the rapidity of germination and uniformity. If moisture is limited, a mixture of the two types is recommended. This gives a viable reserve should early germinating seedlings die due to environmental stress (Hodges et al., 1982)

Aeschynomene is typically seeded at the onset of the rainy season (June 1 to July 1) (Hodges et al., 1982). Germination of hulled seed may be rapid but seedling growth is somewhat slow. Competition from weeds and rapidly growing grasses must be minimized at this time. (Hodges et al., 1982; Bishop et al., 1985). If seeding into a conventionally tilled area, aeschynomene seed should be rolled or disked into the soil to no greater depth than 3 cm, otherwise emergence is hindered (Hanna, 1973). For sustained Aeschynomene production, liming to pH 5.5 to 6.0 and low levels of P and K are recommended, especially on land not previously cultivated (Hodges et al., 1982; Bishop et al., 1985).

Studies in Florida have shown that aeschynomene can be high in forage quality and that maximum forage production occurs during the time when the grass quality is often too low to sustain high levels of animal performance (Ocumpaugh and Dusi, 1981; Gildersleeve, 1982; Sollenberger, 1985). Paul (1951) described the nutritive value of aeschynomene as being comparable to that of alfalfa, but indicated it was less palatable. Kalmbacher and Mislevy (1978) obtained a crude protein concentration of 185 g kg^{-1} DM from plants cut at 30 cm, and 151 g kg^{-1} DM when cut at 90 cm. When cut at 30 cm throughout the season CP was 175 g kg^{-1} DM averaged over the whole season, and IVDOM was 700 g kg^{-1} OM (Mislevy et al., 1981). Bishop et al., (1985) found crude protein and phosphorous values of 160 g kg^{-1}

and 120 g kg^{-1} respectively in the leaves, and 60 g kg^{-1} and 0.3 g kg^{-1} in the stems. Gildersleeve (1982) found the leaf plus seed fraction averaged 250 g CP kg^{-1} DM, and 750 g DOM kg^{-1} OM. Mineral status of 30 to 90 cm regrowth of aeschynomene was determined by Kalmbacher et al. (1981) who concluded that, with the exception of Cu, the needs of growing calves and yearlings should be met by this forage without supplementation.

A compiled bibliography on diseases of the genus Aeschynomene included viral diseases such as tobacco streak virus and legume little leaf disease; fungal pathogens in the genera Uredo, Colletotrichum, Cercospera, and Physoderma (Sonoda and Lenne, 1979; Kretschmer and Bullock, 1980). Armyworm defoliation has been reported at Ona (Kalmbacher et al, 1981). Kretschmer and Bullock (1980) report leaf and stem feeding by a looper (Selenis monotropa Grote).) and minor damage due to a microlepidopteran leaf binder. Snails are major pests to seedlings in grass sods (Kalmbacher et al., 1979).

Effects of Root-Knot Nematodes on Aeschynomene

Minton et al. (1967) examined aeschynomene's responses to the common nematode species using a 1-5 scoring system where 1 = no galls or few, and 5 = heavy galling. The results were as follows: aeschynomene was fairly tolerant of M. incognita var. incognita, (mean gall score of 1.5); intermediately susceptible to M. javanica, M. arenaria, and

M. hapla (mean gall scores of 2.8, 3.0 and 2.2 respectively); but decidedly susceptible to M. incognita var. acrita (mean gall score of 4.2).

In field trials, Kretschmer et al., (1980) concluded that Florida common was resistant to southern root-knot nematode (Meloidogyne incognita). Rhoades (1980), though also concluding that aeschynomene was resistant to M. incognita, found that some nematodes developed and produced eggs. Though, Rhoades (1980) found DM yields not significantly reduced; gall scores were significantly correlated with successive harvests of DM yield (r^2 increased from -0.43 to -0.78 from the first to the third harvest).

Pasley (1981) examined the responses of several A. americana genotypes to M. incognita. He found both resistant and susceptible ecotypes but cautioned that not all sources of Florida common aeschynomene were resistant. Pasley developed a method of examining the responses in the greenhouse, which was correlated with the field results and losses in dry matter.

Taylor (1984) found aeschynomene moderately susceptible to M. incognita due to the amount of galling and egg production. Aeschynomene appeared to be resistant to M. arenaria, although a few eggs were found. It was only moderately tolerant of M. javanica in terms of galling, but egg production was not significantly different from zero.

The literature on the response of aeschynomene to root-knot nematodes has some conflicting points of view. In some cases aeschynomene was resistant and in others it was more susceptible to root-knot nematodes. It may be that different aeschynomene species and genotypes were used, and each type gave different responses. Or it may be that the nematode species used were not correctly identified and the aschenomene genotype used responded differently to each species.

Root-Knot Nematodes (Meloidogyne spp.).

Taxonomy

From 1879 to 1949 root-knot nematodes were included in the genus Heterodera (Wouts, 1979). In 1949, Chitwood redefined the genus Meloidogyne and differentiated it from Heterodera by the pattern of striations on the posterior region of the mature female (perineal pattern), the shape of the stylet knobs, the length of stylets, the shape of the mouth region, and the distance between the mouth and excretory pore.

Meloidogyne is part of the Phylum Nemata, belonging in the Class Secernemtea, Order Tylenchida, Superfamily Tylenchoidea and Family Meloidogynidae (Wouts, 1979). The first species identified were Meloidogyne exigua, which was first described by Goeldi in 1887, and is the type species. M. javanica was first reported by Treub in 1885; while M. incognita was seen by Kofoid and White in 1919, and

separated into varieties incognita and acrita by Chitwood in 1949. M. arenaria was described by Neal in 1889 and reclassified by Chitwood in 1949. M. hapla was also described by Chitwood in 1949 (Chitwood, 1958; Wouts, 1979).

In 1979 there were some 36 species ascribed to the genus and some 51 in 1982 (Hirschmann, 1982). M. javanica, M. incognita, M. hapla, and M. arenaria make up 95% of the world population of root-knot nematodes, and are collectively capable of attacking virtually every crop species grown (Sasser, 1979a, 1979b; Carter and Sasser, 1982). Estimates of worldwide crop damage attributed to Meloidogyne species range from 5 to 15%. In reality over 80% of the damage is restricted to the subtropical and tropical regions of the world (Sasser, 1979), making the root-knot nematode species the fifth most important pest in the world (Taylor and Sasser, 1978; Carter and Sasser, 1982).

M. hapla, the Northern root-knot nematode, makes up 8% of the world populations (Carter and Sasser, 1982) and is primarily known for its effects on carrots, turnips, sugar beet, and a host of other vegetables in the northern hemisphere (Franklin, 1979a, 1979b). Though the overall economic losses due to this species are not great, in the individual case they can be serious.

M. arenaria, known as the peanut root-knot nematode, makes up 7% of the world population and has two races: race 1 and race 2. The former attacks peanuts and it is more common in Florida; race 2 does not parasitize peanuts and it is more common world-wide (Carter and Sasser, 1982). M. arenaria, as the common name suggests, is primarily found on peanuts in the southern U.S., but is also known as a serious problem in vegetables, in the subtropics and Mediterranean climates (Lamberti, 1979).

M. javanica, the javanese nematode as it was described by Chitwood (1958) from samples of sugar cane from Java, makes up 31% of the world populations (Carter and Sasser, 1982). It is the second most widespread species of root-knot nematodes in the tropical and subtropical regions of the world. Vegetables such as tomato, beans, melons, and squashes are most seriously affected, but other crops including sugar cane, tobacco, soybean, peanut, forage legumes, and rice may also be severely damaged. Ornamentals, such as carnations, suffer as well as tree crops (peach and almond) (Lamberti, 1979).

M. incognita, the southern root-knot nematode, is the most widespread root-knot nematode species comprising 52% of the world populations. It has been divided into four races which according to Carter and Sasser (1982) comprise the following percentages of samples identified from worldwide collections:

race 1 = 72% (var. "incognita")

race 2 = 13%

race 3 = 13% (var. "acrita")

race 4 = 2%

The races of M. incognita can be distinguished by the Host Differential Test, but also somewhat by the perineal patterns. Most crop species, vegetable, row crops and ornamentals, can be attacked by at least one of these races. The species is generally only restricted only by environmental constraints, such as soil type and temperature. It is primarily found in the subtropics, Mediterranean regions and tropics.

The Life Cycle of Root-Knot Nematodes.

The life cycle of the genus Meloidogyne starts in the egg (Guiran and Ritter, 1979). Eggs are laid at the one-cell stage and cell division starts after a few hours. Embryogenesis is accelerated with higher temperatures and retarded by low temperatures; it can be between 9 and 31 days (Minton, 1962; Taylor and Sasser, 1978). Eggs are deposited in a gelatinous matrix which is attached to the posterior of the female.

The first molt takes place in the egg; the second stage juvenile (J2) survives on its food reserves. The J2 hatches by thrusting the stylet repeatedly at one end of the egg shell, until it is split. For tropical species, such as Meloidogyne incognita, M. arenaria and M. javanica,

hatching is spontaneous, under the optimal temperature and humidity conditions. Hatching chemical stimuli have been suggested but as yet none has been found. Hatching is suppressed by unfavorable or stressful conditions, such as high or low temperatures, low humidity, lack of oxygen or high osmotic pressure. If the stress is limited, hatching will proceed rapidly once the stress has been removed. There appears to be a dormancy or diapause mechanism, whereby the J1's and J2's can overwinter or wait out a stress period. Diapause enables the nematode to persist in the soil for weeks, months or longer time periods.

Once hatched, the J2 must find a host root to penetrate. It is at this stage that the nematode is most vulnerable, without the protection of the egg mass matrix or a host root. The J2 migrate to the root tissue and will explore it. There has been some evidence of a chemical attractant to guide the juvenile to the root, but only in a few specific plant species. Once the host has been penetrated, development proceeds fairly rapidly, but that depends on environmental conditions, including the host type, and status.

The J2 eventually comes to rest with its head in the stele and body in the cortex (perpendicular to the stele). The stylet pierces the cell walls and esophageal gland secretions are injected. These secretions cause cell enlargement (hypertrophy), which leads to the development

of syncytia or giant cells, and cell division (hyperplasia), which often results in the formation of a gall, but not necessarily. While the giant cell is forming the juvenile is enlarging, and becomes flask-shaped. The oesophageal glands enlarge, the cells of the genital primordia divide and six rectal glands, which secrete the ovisac, develop in the posterior end of the female larva (Taylor and Sasser, 1978).

The larvae then undergo three further molts (the third and forth juvenile stages are rather short) (Taylor and Sasser, 1978). The last molt results in the adult form, either male or female, depending on the conditions. The male is filiform and the female enlarges to become pyriform. After reproduction, the female secretes a gelatinous matrix into which she extrudes a large number of eggs, usually 500-1000 (Guiran and Ritter, 1979). After a few generations, the gall may contain a number of females of various ages.

The span of the life cycle varies with the species and the environmental conditions, especially temperature. Overall the threshold of development is considered to be about $9^{o}C$. The rate of development increases with temperature, up to about $28^{o}C$, after which it is retarded. The optimal temperature depends on the species; more temperate climate species have lower optima, and tropical species have higher optimal temperatures (Griffin and Elgin, 1977).

The Meloidogyne genus is generally parthenogenic in nature, that is without male participation in reproduction (Triantaphyllou, 1979). M. arenaria, M. incognita and M. javanica generally have obligatory mitotic parthenogensis which maintains the diploid number. When males are not needed, i.e. under favorable conditions, they do not develop. Under stressed conditions, males develop as they need fewer resources and sperm is used to increase genetic variation (Papadopoulou and Triantaphyllou, 1982).

Plant Damage Due to Root-Knot Nematodes

Nematode damage usually results in water and nutrient deficiencies. The root is often stunted or stubby, lacking root hairs, and is generally less efficient. The above ground parts may not show any signs under light infection, but if the plant is severely infected, it may be stunted, chlorotic, wilted, and prematurely senescent. An important result of nematode infection is the predisposition or the plants vulnerability to attack and infection by other pathogens. The nematode weakens the plant and creates an entrance for other organisms. Other secondary organisms commonly associated with root-knot nematodes are Phytophthora, Verticillium, Helminthosporium, Pythium, and Aspergillus (Taylor and Sasser, 1978). For example, resistance in tomato and tobacco to Fusarium spp. is broken down when the plant is infected with M. incognita (Taylor and Sasser, 1978).

Root-Knot Nematode Control by Resistant Cultivars

The control of root-knot nematodes can be achieved by various methods. Chemical control may not always be possible due to practical difficulties, expense of repeated application, and governmental regulations which have reduced the number of chemicals available. Control by the use of natural predators (i.e. bacteria and viruses), though researched and theoretically feasible, has yet to be achieved on a commercial scale. Crop rotation can be an effective method but requires long time intervals between susceptible crops due to the longevity of the nematodes. The rotational crop should also have some economic value as well as resistance to root-knot nematodes. The most desired method of control would be by resistant cultivars, as nematode resistant or tolerant cultivars will out-yield susceptible ones in nematode-infested fields. Also the need for other control such as fallowing, crop rotations, or soil treatments with nematicides is minimized or eliminated. The resistant cultivars generally inhibit the nematode reproduction, and therefore reduce the soil populations which may permit the cultivation of crops which are susceptible in following seasons. Fassuliotis (1982) estimated that a cultivar resistant to M. incognita and M. javanica could be grown successfully on 83% of the root-knot infested areas of the world. With resistance to M. arenaria it could increase to 88%.

Though cultivars have been developed with root-knot nematode resistance, only a few crop species are included. Sasser and Kirby (1979) have listed cultivated species and varieties which have resistance to at least one Meloidogyne species. The forage and grain legume cultivars included by Sasser and Kirby with resistance or tolerance to at least one species of root-knot nematode are alfalfa, bird's-foot trefoil, soybean, peanut, cowpea, crown vetch, lima bean, pea, subterranean clover, vetch, and common bean. On the whole these cultivars were not specifically bred for resistance, and thus the genetics and inheritance of that resistance are largely unknown.

The literature on root-knot nematode host plant resistance is extensive but tends to be mainly concerned from the point of view of the nematode. Review articles on nematode resistance include Hare (1965), Sasser (1972, 1982), and Fassuliotis (1979, 1982). There is very little information as to the inheritance of the plant response and none on the responses in tropical forage legumes. Therefore in order to form a basis for comparison we shall have to discuss the information available on other legumes.

Cowpeas (Vigna unguiculata (L.) Walp.)

Hare (1959) found resistance to M. incognita in cowpeas to be controlled by one gene and that resistance was dominant. The line 'M455' (resistant) was crossed with "Brown Sugar" (susceptible). The third filial (F3)

generation was examined for its response. Thirty eight, out of eighty lines, segregated in a 2.3:1 ratio of resistant to susceptible. Amosu and Franckowiak (1974) found similar results on a larger scale with four cowpea cultivars crossed and the second filial (F2) generation measured. Frey and Dukes (1980) examined the parents, first filial generation (F1), F2, F3 and backcross generations from a resistant x susceptible cross and found one dominant gene controlling resistance to M. incognita, M. javanica and M. hapla.

Lima beans (Phaseolus lunatus L.)

McGuire et al (1961) examined the inheritance of response to M. incognita var. acrita in Lima beans. Two susceptible lines were crossed with two resistant lines and the F2 and F3 generations were tested. They reported low heritabilities and suggested that only a few major genes controlled the trait. However, low heritabilities do not necessarily imply only a few major genes, nor is a heritability estimate appropriate for a qualitative trait.

From a resistant x resistant cross some susceptible F2's were recovered, which suggested that the sources of resistance in the parents were not the same. Allard (1974) used recurrent backcrossing between a resistant line (L9) and a susceptible to transfer the trait and release resistant cultivars: 'Westan' and 'Ventura N', with moderate and high resistance to M. incognita.

Beans (Phaseolus vulgaris L.)

Barrons (1940) found resistance to M. incognita in common beans to be controlled by the interaction of two recessive genes. 'Alabama 1' (resistant) was crossed with a susceptible line and gave all galled F1's. Subsequent generations (F2's and F3's) segregated for two independent recessive genes. Blazey et al. (1964) did a complete four way diallel between two susceptible and two resistant lines, including the lines used by Barrons (1940). The results were similar and intermediate responses were deemed a result of incomplete dominance. Still working with M. incognita, Hartmann (1971) crossed 'Alabama 1' with the susceptible 'Hawaiian Wonder' and found the F2 ratio of resistant to susceptible to be greater than 1:15 and a larger proportion in the F3. He concluded that resistance was controlled by at least three recessive genes, which were equal in action and a certain minimum number were required for susceptibility. He also noted that the gene models worked out were based on the number of galls and the gall sizes, which tended to make the scoring somewhat variable and arbitrary.

Alfalfa (Medicago sativa L.)

Work on alfalfa started in the sixties with Goplen and Standford (1960), who concluded that resistance to M. hapla and M. javanica in 'Vernal' was controlled by one dominant gene for each. The genes appeared to be related and

inherited tetrasomically. Hunt et al. (1969) found similar results and found that they could create a nematode resistant cultivar with pea aphid, spotted aphid and bacterial wilt resistance maintained. Sullivan et al. (1980) stated that alfalfa resistance to M. hapla was in fact controlled by at least two loci which segregated tetrasomically. They showed that at least two dominant alleles had to be present at both loci for a genotype to show resistance, and that the loci should be symbolized as Rk_1 and Rk_2.

Sericea Lespedeza (Lespedeza cuneata (Dumont) G. Don.)

Hanson et al. (1954) studied the resistance to M. incognita and M. incognita var. acrita in the F5 generation from a susceptible x resistant cross of sericea lespedeza. They concluded that the variation was mostly genetic and suggested that selection should be between families. They also found a high positive phenotypic correlation between the resistances to both nematodes. They hypothesized that the genes for resistance may be similar or the same, each conferring resistance to the other and therefore only one need be selected for. Adamsom et al. (1974) found resistance to M. hapla in sericea lespedeza was controlled by a single dominant gene.

Soybean (Glycine max L. Merrill)

Hinson and Hartwig (1964) crossed 'Jackson' (resistant to M. incognita) with D49-2491, and carried out six

generations of selfing to create 'Bragg' as the first root-knot nematode resistant soybean cultivar. 'Centennial', also resistant to M. incognita, was developed from an F5 line which was derived from a series of crosses: ('Hill' x (D498-2491 x 'Jackson')) x 'Pickett 71' (Hartwig and Epps, 1977). The actual inheritance or gene action of this resistance in soybeans has not been completely determined as yet.

Forage Legume Germplasm

Baltensperger et al. (1984) identified root-knot nematode as a main limiting factor in production of alyceclover (Alysicarpus vaginalis (L.) DC.). Baltensperger et al (1983) also reported considerable variation among species in various traits including nematode galling to M. javanica and M. incognita, but found that no one line was resistant to both nematode species. Taylor et al. (1986) also examined the responses to root-knot nematode species in Alysicarpus. They found variation did exist but no one genotype was highly resistant to more than one species of root-knot nematode. The broad sense heritabilities were high, indicating that the variation was genetic. They also pointed out that endowing a variety with resistance would enhance its value as a crop, making it usable as a rotation crop which reduces nematode populations and making areas productive which had hitherto been limited due to the nematodes.

Quesenberry et al. (1983) screened 12 desmodium (Desmodium heterocarpon (L.) DC.) plant introductions in a greenhouse trial. Most were highly susceptible, especially to M. javanica and M. arenaria, but a few lines expressed some tolerance to M. incognita. Kretschmer et al. (1980) and Taylor (1984) found some plants so severely affected they actually did not survive in the field. Lenne (1981) reported damage caused by M. javanica on Desmodium spp. in South America. Though the root-knot nematode distribution throughout the pasture sites was limited, its pathenogenicity was so severe on the Desmodium lines tested that she suggested working to develop resistance.

Hairy indigo (Indigofera hirsuta L.) is popular as a forage legume partly due to its nematode resistance. But there have been some reports of susceptibility to the four main species of root-knot (Good, 1972). Rhoades (1976) found vegetable crop yields improved if hairy indigo had preceded it in the field, but cautioned against the use of highly susceptible crops following hairy indigo as it seemed somewhat inconsistent in response. Taylor (1984) found hairy indigo to be moderately galled by M. incognita, M. arenaria and M. javanica but that egg production was very low.

Showy crotalaria (Crotalaria spectabilis Roth.) seemed to be tolerant of the main root-knot species listed above; although some juveniles had penetrated the roots they were

unable to mature. It failed to reduce populations in the field (Good et al. 1965; Soffes, 1981). Taylor (1984) found it to be resistant.

Pigeon pea (Cajanus cajan L.) seemed to be resistant to M. incognita and M. javanica but was susceptible to M. arenaria (Minton et al., 1967; Taylor 1984).

Because Aeschynomene americana has considerable potential as a forage legume, and a plant breeding program has been initiated in Florida to improve its agronomic traits and expand its uses. To date there is relatively little known about the species as a whole and as a crop. An ecotype selection has been released, but no genetically improved cultivars have been developed and released.

Root-knot nematodes are endemic to the tropics and because Aeschynomene is largely a tropical genus, any variety used would need to have resistance to root-knot nematodes.

Root-knot nematodes are a destructive insect pests on crop species. These nematodes may attack any crop and are a growing problem in the tropics and subtropics. The life cycle of the nematode takes place largely in the plant roots, making chemical control difficult. From the information known, it seems that the most effective method of control is by host plant resistance.

The literature on root-knot nematode resistance in legumes generally suggests that resistance is usually

controlled by a few major genes for each nematode. Though not outlined here, the results seen in other crops such as tomato, tobacco, and cotton also indicate that resistance is controlled by a few major genes.

CHAPTER III
NORTH CAROLINA HOST DIFFERENTIAL TEST

Introduction

Genetic resistance to a pathogen may be controlled by any number of genes and genetic systems. Genetic resistance to one particular species or race does not necessarily confer resistance to other species and races. When a plant is infected with more than one species or race of a pathogen the respective genetic systems may well be interacting and confounding each other. Usually each genetic system is individually examined as it is easier and more accurate to measure each system separately. Once all the genetic factors are known they can be combined into one system imparting resistance to all the species and races concerned.

There are four root-knot nematode species which are collectively capable of attacking virtually every crop species grown and are therefore considered the major plant-parasitic root-knot nematodes (Carter and Sasser, 1982). These species of root-knot nematodes were first identified and classified by Chitwood (1958). He based the identification of the individual species primarily on the pattern of striations on the posterior region of the mature female (perineal pattern). The International Meloidogyne

Project (Raleigh, North Carolina) developed a means of further identifying the various species and races known as the "North Carolina Host Differential Test" (Taylor and Sasser, 1978).

Pasley (1981) examined the responses of 11 Aeschynomene americana germplasm lines to several species of root-knot nematodes. Subsequently, K. H. Quesenberry developed three families (parents, F2's, and F3's) from crosses among lines which Pasley had shown to be resistant and susceptible. These were examined in a preliminary (unpublished) experiment in early 1983 and found to be nearly all susceptible. A possible explanation of these results was that the Meloidogyne population used had become contaminated with another species. If this was the case, the genetic systems may have been counteracting each other in such a way so as to break down resistance to one or both species.

Thus the object of this investigation was to identify and characterize the University of Florida Meloidogyne incognita population using the North Carolina Host Differential Test combined with an examination of the perineal patterns.

Materials and Methods

The test was conducted in the greenhouse during November 1983 through January 1984 with a temperature range of 21° to $36^{\circ}C$. Five plants for each indicator plant

species were used, and each plant was grown in a 15 cm clay pot in methyl bromide fumigated soil.

The plants were inoculated on 23 Nov., 1983 with 10,000 eggs per pot via a re-pipetting syringe. The inoculation of the plants was with second stage juveniles ("J2") collected from infested Rutgers tomatoes (Lycopersicon esculentum Mill.). The tomato plants were grown in sterilized soil and harvested when the egg masses were light brown, usually at least 45-55 days after inoculation, depending on the temperature. The roots were cut into pieces 2 to 3 cm long and placed in a 500 mL container with 200 mL of 0.5% sodium hypochlorite (NaOCl) solution. They were then stirred vigorously by hand for about 3 minutes. The liquid suspension of eggs and roots was then poured through a 200-mesh (openings 0.149-0.074 mm) sieve and a 500-mesh (0.028 mm openings) sieve. The eggs were separated from the egg mass by 0.5% NaOCl and retained on the 500-mesh sieve. The 0.5% NaOCl solution was rinsed off, thoroughly, by a slow stream of cold tap water. The eggs were then washed into a 1 L container. The concentration of eggs mL^{-1} was determined by filling the container to 1 L with cold tap water and counting the number of eggs. The eggs were counted in 3 or 4 samples of 1 mL each using a 'Peter's 1 mL eelworm counting slide' (Hawksly Manufacturing; London G.B.). While sampling and inoculating, the eggs were kept in continual motion by a magnetic stirrer.

The original container and roots may be washed twice to collect additional eggs. When very large numbers were needed the whole process was repeated.

The plants were fertilized regularly and watered daily. The cultivars used were:

1. tobacco (Nicotiana tabacum L.) cv "NC 95";
2. cotton (Gossypium hirsutum L.) cv "Deltapine 16;
3. pepper (Capsicum frutescens L.) cv. "California Wonder";
4. watermelon (Citrullus vulgaris Schrad.) cv. "Charleston Grey".
5. peanut (Arachis hypogea L.) cv. "Florrunner";
6. tomato (Lycopersicon esculentum) cv. "Rutgers".

(Seeds were obtained from the Nematology Laboratory, University of Florida and the International Meloidogyne Project at Releigh, North Carolina).

The plants were harvested 49 days after infestation (27 Jan., 1984). The roots were washed free of soil and stained with Phloxine B (15 mg L^{-1}) for 15 minutes. The gall and egg mass scores were obtained, where: 0 = no galls or egg masses; 1 = 1 or 2; 2 = 3 - 10; 3 = 11 - 30; 4 = 31 - 100; and 5 = >100 or the plant is dead.

Taylor and Sasser (1978) recommended checking a Host Differential Test with a check on the location of the female excretory pore (perineal pattern). The infected roots were placed in a 0.9% sodium chloride solution and the females dissected out from the root under a dissecting

microscope. The female was then placed in 45% acetic acid in a plastic petri dish and the posterior end cut off with a sharpened needle. The perineal pattern is trimmed and transferred to glycerine on a microscope slide and a cover slip applied. The perineal patterns are formed by the expansion and alteration of the larval body and retain the lateral lines, the tail tip and phasmids (See figure 3-1). A sampling of 4 or 5 adult females were dissected out from three plants of each species, when they were present. The perineal patterns were examined by Mr. F. E. Woods, of the Nematology Laboratory, University of Florida, and confirmed by Mr. A. L. Taylor, and identified from the descriptions given by C.I.H. (1973a, b, and c) and Taylor and Sasser (1978) which are given on Figure 3-1.

Results and Discussion

The International Meloidogyne Project found that the four races of Meloidogyne incognita, and the two races of M. arenaria were distributed world wide and can be distinguished by their reactions in a standardized test, known as the North Carolina Differential Test (Taylor and Sasser, 1978). Race 1 of M. incognita did not attack cv. NC 95, tobacco nor Deltapine 16, cotton. Race 2 attacked the tobacco cultivar but not the cotton. Race 3 attacked the cotton but not the tobacco cultivar. Race 4 attacked both. Race 1 of M. arenaria attacked Florrunner peanut

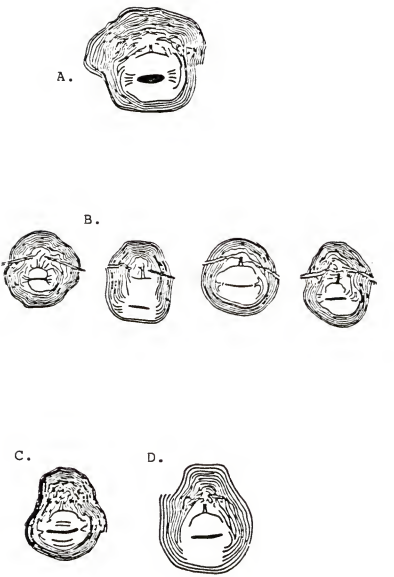

Figure 3-1. Diagrams of Posterior Cuticular Patterns of Meloidogyne spp. Females. A. M. arenaria, B. M. javanica, C. M. incognita var acrita, and D. M. incognita var incognita. Source: Taylor and Sasser, 1978.

and race 2 did not.Thus by the differences seen in a Host Differential Test the various species and races could be identified.

The results of the this experiment in this case indicated that the Meloidogyne population used contained at least two species of root-knot nematode, M. javanica and M. incognita (See Tables 3-1 and 3-2). The results in terms of gall and egg mass scores of the host plant species (Table 3-1) did not indicate which race of M. incognita was present; race 3, race 4 or race 1. The results given on Table 3-2 combined with the knowledge of the species' and races' distributions were used to determine the identification of the M. incognita race present. As race 4 of M. incognita comprises about 2% of the M. incognita found world wide (Carter and Sasser, 1982) and is not commonly found in Florida, it was considered unlikely that it was found in the population. The perineal pattern found on the females from the Deltapine 16 cotton agreed with those in the published description of M. incognita acrita type or race 3 (Figure 3-1). Perineal patterns of race 1 (or var. incognita) and race 4 were not seen. Therefore it was concluded that the root-knot population contained both M. javanica and M. incognita race 3.

Based on these results and host plants, a new population of M. incognita race 3 was established from eggs removed from females found on the Deltapine 16 cotton

Table 3-1. Observed and Expected Reactions from the North Carolina Host Differential Test.

Meloidogyne species and races	Differential Host Plant Cultivars[+]					
	Tobacco	Cotton	Pepper	Watermelon	Peanut	Tomato
U.F. population *M. incognita*	+[++]	+	+	+	-	+
Race 1	-	-	+	+	-	+
Race 2	+	-	+	+	-	+
Race 3	-	+	+	+	-	+
Race 4	+	+	+	+	-	+
M. arenaria						
Race 1	+	-	+	+	+	+
Race 2	+	-	-	+	-	+
M. javanica	+	-	-	+	-	+
M. hapla	+	-	+	-	+	+

+ Tobacco (*Nicotiana tabacum*) cv. NC 95; cotton (*Gossypium hirsutum*) cv. Deltapine 16; pepper (*Capsicum frutescens*) cv. California Wonder; watermelon (*Citrullus vulgaris*) cv. Charleston Grey; peanut (*Arachis hypogea*) cv. Florrunner; tomato (*Lycopersicon esculentum*) cv. Rutgers.

++ +/- identification by presence or absence.

Table 3-2. Gall and Egg Scores, and Perineal Patterns of the Plants Infested with the University of Florida Meloidogyne incognita Population.

Plant Species	N	Gall Score	Egg Score	Perineal pattern	Comments
Tobacco: NC 95	5	4.5	4.5	M. javanica	Large galls
Cotton: Deltapine 16	5	3.5	3.5	M. incognita acrita type	Profusion of egg masses
Pepper: California Wonder	5	3.5	3.5	M. incognita acrita type	Advanced and abundant
Watermelon: Charleston Grey	5	4.5	4.5	M. incognita	Plants stunted
Peanut: Florrunner	4	Ø	Ø		Plants healthy
Tomato: Rutgers	5	5	5	M. incognita acrita type	Heavily infested

(which only supports M. incognita race 3). Five Rutgers tomato plant seedlings were inoculated with five egg masses each, which averaged between 500 and 600 eggs per egg mass. The identification was double checked by examining the perineal patterns of each of the reproducing females.

As the cotton plant does not create a large root system, it was considered not suitable to use as a host to create large nematode populations but a means of maintaining a pure population. The population was maintained and increased by inoculating Rutgers tomato seedlings, and leaving them to grow for 60 days, which allows for at least 2 cycles of reproduction under normal conditions (Taylor and Sasser, 1978).

In order to have a complete set of populations of the major root-knot nematodes which are prevalent in the more tropical regions of the world, a population of M. incognita race 1 was also established. M. incognita race 1 was started from eggs which were furnished by the International Meloidogyne Project at Raleigh, North Carolina.

The North Carolina Host Differential Test combined with the perineal patterns proved an effective method of identifying the species and races present. Once having identified the species and races, pure populations were established. Thus, as of March 1984, the Meloidogyne incognita populations were considered pure and uncontaminated.

CHAPTER IV
PARENT EVALUATION AND SELECTION

Introduction

In order to study the genetics of a particular trait, a thorough investigation needs to be made of the parental material to be used. The first step is to determine the range that exists in the trait, i.e. does resistance exist in the population. In conjunction with this first step, one must also be able to recognize different genotypes by their responses. The second step, as important as the first, is to determine if the trait is to some extent genetically controlled. This information, coupled with the botanical information as to the reproduction of the species, determines the type of genetic analysis and the subsequent interpretations that can be made.

Pasley (1981) evaluated responses of eleven aeschynomene lines to M. incognita. He found variation among genotypes but did not report variation within genotypes except in the case of 55, which though described as resistant showed some signs of galling. He also developed the technique which was used in these studies, with one modification.

Objectives of the two experiments were (1) to define the range, especially the extremes, and recognize differences between the responses of the genotypes; (2) to identify genotypes that represented wide ranges in responses; and (3) select a group of appropriate parents for further work.

Methods and Materials

Experiment 1 was conducted in order to examine responses of 73 Aeschynomene americana plant introduction lines. These plant introductions were evaluated their responses to the following nematode species: Meloidogyne incognita race 3, M. arenaria race 1, and M. javanica. The plant introductions were examined with a factorial design with two replications of three plants each.

Experiment 2 was conducted to test the consistency of the response of seven selected lines. These lines were selected from the whole population based on their performance in previous studies. The lines were numbered: 55, 167, 173, 177, 189, 232 and 239. These seven were infested with the following nematodes species: Meloidogyne incognita race 1 and race 3, M. arenaria race 1, and M. javanica, in two replications of seven plants each, also making a factorial design.

In both experiments the seeds were scarified, germinated, and planted in 150 cm^3 containers (Conetainers), filled with methyl bromide fumigated

Arredondo fine sand top soil (loamy, silicious, hyperthermic, Grossarenic Paleudult). The plants were grown in the greenhouse, under moderately high temperatures (22 to 37 ^{o}C), inoculated with cowpea type Rhizobium and watered daily. Two weeks after they were planted, they were inoculated with 10 nematode eggs per cm^3 of soil (1500 eggs per plant). The eggs were amassed by the method described in Host Differential Test (Chapter III). The nematode eggs were maintained in an aqueous suspension, by continuous stirring with a magnetic stirrer and injected into the soil around the seedlings in two aliquots of 3 mL each (250 eggs mL^{-1}) each via a repipetting syringe.

Eight weeks after infestation, the plants were removed from the containers and washed free of soil. The roots were immersed in an aqueous solution of Phloxine B stain (15 mg L^{-1}) for 15 minutes to stain the gelatinous matrix of the egg masses. Root galling and egg mass production were rated using the 0 to 5 scale described in Chapter III.

Statistical Analysis

Experiment 1. Germplasm Evaluation.

The model used to analyze the 73 lines was considered to be:

$$y_{ijkl} = u + a_i + b_j + ab_{ij} + c_k + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkl}.$$

where:

y = response (y_1 = gall score and y_2 = egg mass score)

u = the overall mean.

a = the effect of the ith nematode (N) species, where i = 1,2,3,4.

b = the effect of the jth genotype (G), where j = 1,..,73.

c = the effect of the kth replication (R), where k = 1,2.

e = remainder, where l = 1,2,3. for the number of plants per rep.

The model was considered mixed, with genotypes and nematodes considered as random effects and replications as fixed. The analysis was made using SAS (1985). The F values were calculated by hand, using the error terms given on Table 4-1. Where the F-ratio did not follow the F-distribution and an approximation was necessary; Satterthwaite's Rule was used to calculate degrees of freedom (Cochran and Cox, 1957). Nematodes were found to vary significantly for both dependent variables. A reduced model was analyzed for each nematode species (Table 4-2, 4-3, and 4-4).

The variances calculated from the analyses are given on Table 4-5, and were used to calculate "Broad sense" heritabilities, as defined by Falconer (1981), where H^2 = Genotypic variance/Total Phenotypic variance. Over all three nematode species, replications were considered fixed, and hence were not included in the H^2 calculation; nematodes were considered as random environmental effects on the plant genotypes.

Table 4-1. Analysis of Variance for Gall and Egg Scores for the Complete Model+.

Source	d.f.	Gall Scores			Egg Mass Scores			Error Term
		Mean Squares	F Value	PR>F	Mean Squares	F Value	PR>F	
Replicate	1	6.07	3.48	0.05	2.26	0.53	0.9	MS:(RG) + (NR) - (NGR)
Nematode	2	302.59	128.22	0.00	249.09	89.60	0.00	MS:(NG)
N x R	2	1.76	3.08	0.1	4.37	4.75	0.05	MS:(RNG)
Genotype	72	18.52	7.84	0.00	20.01	7.19	0.00	MS:(NG)
R x G	72	0.55	0.96	0.9	0.84	0.91	0.9	MS:(RNG)
N x G	144	2.36	9.07	0.00	2.78	9.02	0.00	MS:(error)
R x N x G	143	0.57	2.19	0.00	0.92	3.17	0.00	MS:(error)
Error	1033	0.26			0.29			
Total	1469							

+ Only replications were considered fixed.

Table 4-2. Analysis of Variance[+] for Gall and Egg Mass Scores for M. arenaria.

Source	d.f.	Gall Scores Sums of Squares	F Value	PR>F	Egg Mass Scores Sums of Squares	F Value	PR>F
Replicate	1	9.99	11.78	0.00	12.87	10.75	0.00
Genotype	72	764.21	25.85	0.00	789.38	27.12	0.00
R x G	72	61.04	2.07	0.00	86.16	2.96	0.00
Error	343	140.76			138.67		
Total	488	977.53			1028.473		

R^2=0.85, C.V.=28.37 R^2=0.87, C.V.=33.57
+ All effects considered random.

Table 4-3. Analysis of Variance[+] for Gall and Egg Mass Scores for M. javanica.

Source	d.f.	Gall Scores Sums of Squares	F Value	PR>F	Egg Mass Scores Sums of Squares	F Value	PR>F
Replicate	1	0.12	0.30	0.9	0.53	0.76	0.9
Genotype	72	522.78	43.33	0.00	627.57	36.77	0.00
R x G	72	28.68	2.38	0.00	49.98	2.93	0.00
Error	345	57.80			81.78		
Total	490	609.58			760.17		

R^2=0.91, C.V.=10.70 R^2=0.89, C.V.=4.72
+ All effects considered random.

Table 4-4. Analysis of Variance[+] for Gall and Egg Mass Scores for *M. incognita* race 3.

Source	d.f.	Gall Scores			Egg Mass Scores		
		Sums of Squares	F Value	PR>F	Sums of Squares	F Value	PR>F
Replicate	1	0.75	1.56	0.9	0.05	0.06	0.9
Genotype	72	378.19	25.62	0.00	416.33	22.87	0.00
R x G	72	34.46	2.65	0.00	59.13	3.29	0.00
Error	345	70.73			87.21		
Total	489	486.93			564.00		

R^2=0.85, C.V.=14.13 R^2=0.85, C.V.=29.70

+ All effects considered random.

Table 4-5. Variances and Broad Sense Heritabilities+ for Gall and Egg Mass Scores Over all Species and for Individual Nematode Species.

	Over all species (reps. fixed)	Meloidogyne arenaria	Meloidogyne javanica	Meloidogyne incognita
Gall Score				
Genotypic variance	0.80	1.46	1.02	0.71
Nematode variance	0.62	----	----	----
Replication variance	----	0.02	0.00	0.00
N x R variance	0.00	----	----	----
G x R variance	0.00	0.13	0.06	0.08
G x N variance	0.26	----	----	----
G x N x R variance	0.09	----	----	----
Error variance	0.26	0.41	0.17	0.21
Phenotypic variance	2.03	2.03	1.25	1.00
Broad Sense H^2	0.394	0.719	0.813	0.710
Egg mass Score				
Genotypic variance	0.86	1.46	1.19	0.74
Nematode variance	0.49	----	----	----
Replication variance	----	0.03	0.00	0.00
N x R variance	0.01	----	----	----
G x R variance	0.00	0.23	0.13	0.17
G x N variance	0.27	----	----	----
G x N x R variance	0.18	----	----	----
Error variance	0.29	0.41	0.24	0.25
Phenotypic variance	2.12	2.14	1.56	1.16
Broad Sense H^2	0.405	0.684	0.761	0.637

+ Broad Sense H^2 = VG/VP, where VG = Genotypic variance, and VP = Phenotypic variance = Total variance (random effects).

For individual nematode species, replications were considered random environmental effects.

Experiment 2. Seven Selected Parents.

The data of the seven selected parents were analyzed by SAS (1985) and with the Harvey (1977) programs in order to compare the two systems. The data were ordinal, with a scale from 0 to 5, which suggested that errors may not have been normally distributed. Therefore, analyses were carried out after transforming the scores to natural logarithms, as well as on the original data. Results of analyses of transformed data were the same as for the untransformed data and were not presented.

The mathematical model used was:

$$y_{ijkl} = u + a_i + b_j + ab_{ij} + c_k + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkl}$$

where

y = response (y_1 = gall score and y_2 = egg mass score).

u = the overall mean.

a = the effect of the ith nematode species, where i = 1,2,3,4.

b = the effect of the jth genotype, where j = 1,....7.

c = the effect of the kth side or rep, where k = 1,2.

e = remainder, where l = 1,...,7.

All effects, except for the error, were considered fixed.

The Harvey program used was Model 2. The Harvey program does not permit inclusion of interactions higher

than the second order; thus estimates of sums of squares were obtained indirectly. An initial analysis considered only the main effects and their two factor interactions; a second absorbed the entire model. The sums of squares for the three-factor interaction were obtained by difference. By analyzing the data in this manner, the main effects and the two-factor interactions were estimated ignoring the three-factor interaction. The SAS analysis estimated main effects after adjusting for three-factor interactions.

As the gall score and egg mass score represent two measures of the genotype response they were correlated using both programs.

Results and Discussion

Experiment 1.

Analyses of variance for gall scores and egg mass scores showed little difference between replications (Tables 4-1); there was considerable variability due to genotypes and nematodes (Table 4-5). The individual analyses for each nematode gave similar results (Table 4-2, 4-3, and 4-4); although replications were significant in M. arenaria (Table 4-2). Certain genotypes were found to have different responses to this nematode species, between replications. This indicated that either the seed source was not pure, that some lines may not have been homozygous, or that the inoculum used for that treatment was not pure.

In the Review of the Literature (Chapter II), the responses of aeschynomene to root-knot nematodes were reported to vary among researchers and nematode species. Upon reflection, the discrepancies in the literature may be accounted for by different sources of aeschynomene which may not have been the same genotypes, though all were termed 'aeschynomene common'; these different genotypes may have different responses. It also may be that the nematode species varied among the different evaluations and hence different responses could have been obtained.

Means and variances of the gall and egg mass scores for each of the 73 aeschynomene lines, for each nematode (over both replications) are given on Tables 4-6, 4-7, 4-8, 4-9, 4-10, and 4-11. There was a wide range of responses obtained for each nematode species. The ranking of the genotypes varied among nematode species, as demonstrated by the significant genotype x nematode interaction (Table 4-1) and Duncan's Multiple Range Test (Tables 4-6 to 4-11). As genotypes were always a significant source of variance in each analysis of variance (Tables 4-2, 4-3, and 4-4), it was concluded that there were genetic differences among the aeschynomene genotypes within a nematode species in terms of gall and egg mass scores. Broad sense Heritability (H^2) was calculated in order to give an approximation as to how much of the variation was due to the genetic effects. The H^2 estimates for the individual nematode

Table 4-6. Mean Gall Scores of 73 Aeschynomene lines Artificially Infested with Meloidogyne arenaria.

Genotype	Mean[+]	Genotype	Mean	Genotype	Mean
197	5.00 a	216	2.66 f-i	247	1.50 k-o
168	5.00 a	172	2.66 f-i	249	1.50 k-o
221	4.66 a-b	227	2.66 f-i	259	1.50 k-o
193	4.66 a-b	203	2.66 f-i	201	1.50 k-o
220	4.16 b-c	205	2.60 g-i	200	1.50 k-o
239	4.00 b-c	230	2.50 g-i	246	1.50 k-o
195	4.00 b-c	177	2.50 g-i	170	1.33 k-p
192	4.00 b-c	250	2.50 g-i	187	1.33 k-p
194	4.00 b-c	238	2.50 g-i	255	1.16 l-p
235	3.83 b-d	257	2.40 g-i	178	1.16 l-q
240	3.83 b-d	519	2.16 g-j	171	1.16 l-q
169	3.80 c-d	176	2.16 g-j	251	1.00 m-q
223	3.66 c-e	209	2.16 g-j	199	1.00 m-q
182	3.66 c-e	204	2.16 g-j	186	1.00 m-q
231	3.66 c-e	175	2.14 g-j	252	0.83 n-q
219	3.66 c-e	253	2.00 h-k	245	0.83 n-q
233	3.50 c-f	174	2.00 h-k	198	0.83 n-q
232	3.50 c-f	206	2.00 h-k	188	0.66 o-q
222	3.00 d-g	208	2.00 i-k	179	0.66 o-q
215	3.00 d-g	242	1.83 i-m	248	0.66 o-q
218	2.83 e-g	254	1.83 i-m	189	0.58 p-q
226	2.83 e-h	183	1.66 j-n	196	0.50 p-q
217	2.83 e-h	207	1.50 k-o	55	0.42 q
241	2.83 e-h	214	1.50 k-o	167	0.35 q
				256	0.33 q

+ Means in the same column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05, D.F. = 343, MSE = 0.41). Each mean is based on three plants in two replications.

Table 4-7. Mean Egg Mass Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne arenaria.

Genotype	Mean +	Genotype	Mean	Genotype	Mean
168	5.00 a	241	2.33 j-n	178	1.00 q-w
193	4.60 a-b	205	2.20 j-o	200	0.83 r-w
197	4.50 a-c	176	2.16 j-o	207	0.83 r-w
220	4.16 b-d	217	2.16 j-o	187	0.83 r-w
195	4.00 b-e	519	2.16 j-o	214	0.83 r-w
194	4.00 b-e	177	2.14 j-o	206	0.83 r-w
235	3.83 c-f	253	2.00 k-p	259	0.66 s-w
192	3.83 c-f	230	2.00 k-p	252	0.66 s-w
182	3.66 d-g	175	2.00 k-p	249	0.66 s-w
169	3.60 d-g	203	2.00 k-p	186	0.66 s-w
240	3.50 d-h	216	2.00 k-p	170	0.66 s-w
239	3.50 d-h	209	2.00 k-p	198	0.66 s-w
231	3.50 d-h	218	1.83 l-q	171	0.66 s-w
223	3.33 d-i	250	1.83 l-q	189	0.58 t-w
221	3.33 d-i	257	1.80 l-q	251	0.50 t-w
232	3.28 e-i	204	1.66 m-r	245	0.50 t-w
233	3.00 f-j	208	1.50 m-s	248	0.50 t-w
219	2.83 g-k	242	1.50 n-s	255	0.50 t-w
172	2.66 h-l	254	1.50 n-s	196	0.50 t-w
238	2.50 i-m	201	1.33 o-t	179	0.50 t-w
222	2.50 i-m	246	1.16 p-u	55	0.42 u-w
226	2.50 i-m	247	1.16 p-u	199	0.33 u-w
227	2.50 i-m	183	1.00 q-v	167	0.21 v-w
215	2.33 j-n	174	1.00 q-v	256	0.16 v-w
				188	0.00 w

+ Means in the same column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05, D.F. = 343, MSE = 0.404). Each mean is based on three plants in two replications.

Table 4-8. Mean Gall Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne javanica.

Genotype	Mean +	Genotype	Mean	Genotype	Mean
250	5.00 a	252	4.50 a-d	201	3.66 f-i
205	5.00 a	254	4.50 a-d	251	3.50 g-j
197	5.00 a	226	4.50 a-d	242	3.50 g-j
231	5.00 a	230	4.50 a-d	214	3.50 g-j
241	5.00 a	259	4.33 b-e	204	3.33 h-k
240	5.00 a	174	4.33 b-e	186	3.33 h-k
172	5.00 a	246	4.33 b-e	188	3.16 i-l
257	5.00 a	227	4.33 b-e	199	3.16 i-l
239	5.00 a	206	4.16 c-f	208	3.00 j-m
195	5.00 a	519	4.16 c-f	203	3.00 j-m
219	5.00 a	238	4.16 c-f	176	3.00 j-m
194	5.00 a	216	4.16 c-f	215	3.00 j-m
182	5.00 a	222	4.00 d-g	55	2.92 k-n
232	4.92 a	169	4.00 d-g	175	2.85 k-n
235	4.83 a-b	170	4.00 d-g	196	2.83 k-n
193	4.83 a-b	248	4.00 d-g	178	2.83 k-n
233	4.83 a-b	253	4.00 d-g	218	2.83 k-n
192	4.83 a-b	209	3.83 e-h	177	2.64 l-o
220	4.66 a-c	200	3.83 e-h	255	2.60 m-o
168	4.66 a-c	247	3.83 e-h	179	2.50 m-o
249	4.66 a-c	245	3.83 e-h	198	2.33 n-o
217	4.66 a-c	207	3.66 f-i	189	2.23 o
223	4.50 a-d	183	3.66 f-i	167	1.57 p
221	4.50 a-d	187	3.66 f-i	171	1.16 p-q
				256	0.83 q

+ Means in the same column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05, D.F. = 345, MSE = 0.167). Each mean is based on three plants in two replications.

Table 4-9. Mean Egg Mass Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne javanica.

Genotype	Mean +	Genotype	Mean	Genotype	Mean
182	5.00 a	172	4.16 b-f	248	3.16 h-j
240	5.00 a	259	4.00 c-g	214	3.00 i-k
219	4.83 a-b	238	4.00 c-g	186	3.00 i-k
197	4.66 a-c	206	3.83 d-h	169	3.00 i-k
195	4.66 a-c	249	3.83 d-h	199	2.83 j-l
250	4.66 a-c	205	3.83 d-h	208	2.80 j-l
194	4.66 a-c	222	3.83 d-h	242	2.66 j-m
193	4.50 a-d	246	3.83 d-h	207	2.66 j-m
235	4.50 a-d	170	3.66 e-i	188	2.66 j-m
221	4.50 a-d	519	3.66 e-i	203	2.66 j-m
217	4.50 a-d	168	3.66 e-i	176	2.50 k-n
232	4.50 a-d	254	3.66 e-i	215	2.50 k-n
231	4.50 a-d	174	3.50 f-i	255	2.20 l-o
233	4.50 a-d	253	3.50 f-i	55	2.07 m-o
241	4.50 a-d	216	3.50 f-i	179	2.00 n-o
239	4.42 a-d	187	3.33 g-j	178	2.00 n-o
227	4.33 a-e	245	3.33 g-j	175	1.92 n-o
220	4.33 a-e	200	3.33 g-j	189	1.92 n-o
230	4.33 a-e	209	3.33 g-j	196	1.83 o
223	4.33 a-e	183	3.16 h-j	198	1.83 o
252	4.16 b-f	204	3.16 h-j	177	1.71 o
192	4.16 b-f	251	3.16 h-j	218	1.66 o
226	4.16 b-f	201	3.16 h-j	167	1.07 p
257	4.16 b-f	247	3.16 h-j	171	0.83 p
				256	0.00 q

+ Means in the same column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05, D.F. = 345, MSE = 0.237). Each mean is based on three plants in two replications.

Table 4-10. Mean Gall Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne incognita race 3.

Genotype	Mean +	Genotype	Mean	Genotype	Mean
182	5.00 a	219	3.66 d-g	218	3.00 h-k
168	5.00 a	519	3.50 e-h	259	3.00 h-k
197	5.00 a	233	3.50 e-h	247	3.00 h-k
193	5.00 a	169	3.50 e-h	248	3.00 h-k
192	4.50 a-b	223	3.50 e-h	196	2.83 i-l
257	4.33 b-c	232	3.42 e-h	254	2.83 i-l
239	4.21 b-d	252	3.33 f-i	245	2.83 i-l
205	4.00 b-e	201	3.33 f-i	246	2.83 i-l
187	4.00 b-e	231	3.33 f-i	176	2.83 i-l
240	4.00 b-e	221	3.33 f-i	209	2.66 j-m
194	4.00 b-e	230	3.33 f-i	188	2.66 j-m
183	4.00 b-e	226	3.33 f-i	207	2.50 k-n
179	4.00 b-e	216	3.16 g-j	198	2.50 k-n
170	4.00 b-e	203	3.16 g-j	204	2.50 k-n
186	4.00 b-e	222	3.16 g-j	208	2.50 k-n
195	4.00 b-e	172	3.16 g-j	199	2.50 k-n
220	3.83 c-f	200	3.00 h-k	206	2.33 l-o
217	3.83 c-f	253	3.00 h-k	175	2.21 m-o
241	3.83 c-f	242	3.00 h-k	214	2.00 n-p
250	3.83 c-f	249	3.00 h-k	189	1.92 n-p
215	3.83 c-f	177	3.00 h-k	55	1.85 o-p
235	3.83 c-f	178	3.00 h-k	256	1.83 o-p
251	3.66 d-g	227	3.00 h-k	255	1.50 p
174	3.66 d-g	238	3.00 h-k	171	1.50 p
				167	0.92 q

+ Means in the same column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05, D.F. = 344, MSE = 0.205). Each mean is based on three plants in two replications.

Table 4-11. Mean Egg Mass Scores of 73 Aeschynomene Lines Artificially Infested with Meloidogyne incognita.

Genotype	Mean +	Genotype	Mean	Genotype	Mean
168	5.00 a	172	2.83 d-h	519	2.16 h-l
182	4.33 b	231	2.83 d-h	188	2.00 i-l
197	4.16 b	170	2.83 d-h	249	2.00 i-l
193	4.16 b	215	2.83 d-h	177	2.00 i-l
239	3.92 b-c	183	2.83 d-h	178	2.00 i-l
192	3.83 b-c	247	2.83 d-h	253	2.00 i-l
257	3.50 c-d	252	2.83 d-h	196	2.00 i-l
186	3.33 c-e	233	2.83 d-h	198	2.00 i-l
187	3.33 c-e	201	2.66 e-i	248	2.00 i-l
194	3.33 c-e	217	2.66 e-i	214	1.83 j-m
240	3.16 d-f	227	2.66 e-i	209	1.83 j-m
250	3.16 d-f	218	2.66 e-i	199	1.83 j-m
205	3.16 d-g	222	2.66 e-i	176	1.83 j-m
235	3.16 d-g	230	2.50 f-j	246	1.66 k-n
220	3.00 d-g	221	2.50 f-j	175	1.57 l-o
223	3.00 d-g	203	2.50 f-j	206	1.50 l-o
179	3.00 d-g	251	2.50 f-j	208	1.50 l-o
174	3.00 d-g	259	2.33 g-k	189	1.30 m-o
219	3.00 d-g	242	2.33 g-k	207	1.16 m-p
226	3.00 d-g	200	2.33 g-k	256	1.16 m-p
195	3.00 d-g	238	2.33 g-k	55	1.07 n-q
241	3.00 d-g	245	2.33 g-k	204	1.00 o-q
232	2.92 d-g	216	2.33 g-k	255	0.66 p-r
169	2.83 d-h	254	2.16 h-l	171	0.50 q-r
				167	0.42 r

+ Means in the same column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05, D.F. = 344, MSE = 0.252). Each mean is based on three plants in two replications.

species were higher than the H^2 calculated over all the nematode species (Table 4-5), because for the individual nematode species the interactions were not included in the calculation. Otherwise, the weighted average of the three H^2 estimates would equal the overall pooled H^2. As replications were considered fixed and were not significant, they obviously contributed very little to the over all variance. Therefore the over all heritabilities given on table 4-5 do not include replication in the total phenotypic variance. The high H^2's given for the individual nematodes were also due to the little variance between replications, as well as the variation in the two-factor interactions which were not included. As there was a high proportion of total phenotypic variance due to genetic effects, one may suppose that the trait was controlled by relatively few genes for the individual nematode species. However, the low overall H^2's and the changing ranking of the genotypes to each nematode species suggests that the systems which controlled the responses for the individual nematodes had few genes in common.

The results also indicated that the technique described was suitable for screening large numbers of plants in a small space. The inoculation procedure was simple and scoring could be accomplished quickly. Previous work showed that results, from the application of this technique in the greenhouse, were correlated with field

results (Pasley, 1981; Taylor, 1984). Greenhouse screening, however, was simpler and often resulted in more pronounced differences in plant responses. Staining the egg masses was beneficial in that it highlighted the galling response and made the rating process easier.

The highest scoring nematode species were M. incognita race 3 and M. javanica. This, coupled with their wide tropical distribution, suggested that these two nematode species are of greater concern for a plant breeding program designed to incorporate resistance into A. americana.

Experiment 2.

The analyses of variance for gall and egg mass scores are presented in Tables 4-12, and 4-13. The difference between the Harvey program and with the SAS program is that the Harvey analysis does not adjust the main effects and the two factor interactions for the three-factor interaction. Therefore, differences between the analyses are in the estimates of the effects and the sums of squares of for main effects and the two factor interactions. This is demonstrated in SAS for example, using reduction notation, R SS (N/ R, G, NR, NG, RG, RNG), and in the Harvey program R SS (N/ R, G, NR, NG, RG). This discrepancy is obviously necessary only when the data were unbalanced, because of effects of disproportionality resulting from unequal subclass numbers. If the data were balanced then the two analyses would give the same sums of squares.

Table 4-12. Analysis of Variance of Gall Scores for Experiment 2 with Both the SAS and Harvey Analyses.

Source+	d.f.	SAS			Harvey		
		Sum of Squares	F Value	PR>F	Sum of Squares	F Value	PR>F
Replication	1	1.03	3.32	0.07	1.02	3.31	0.07
Nematode	3	103.33	111.56	0.00	103.68	112.20	0.00
R x N	3	1.68	1.82	0.14	1.53	1.66	0.19
Genotype	6	656.88	354.61	0.00	657.47	335.74	0.00
R x G	6	4.52	2.44	0.03	4.52	2.43	0.03
N x G	18	76.49	13.76	0.00	76.03	13.70	0.00
R x N x G	18	9.44	1.70	0.04	9.44	1.70	0.05
Error	332	102.50			102.50		
Total	387	956.66			956.19		

R^2=0.89, C.V.=23.21 R^2=0.88, C.V.=23.62

Table 4-13. Analysis of Variance of Egg Mass Scores for Experiment 2 with Both the SAS and Harvey Analyses.

Source+	d.f.	SAS			Harvey		
		Sum of Squares	F Value	PR>F	Sum of Squares	F Value	PR>F
Replication	1	0.58	1.98	0.16	0.57	1.95	0.17
Nematode	3	52.22	60.50	0.00	53.54	60.86	0.00
R x N	3	0.42	0.48	0.70	0.38	0.43	0.75
Genotype	6	661.56	376.00	0.00	661.72	376.14	0.00
R x G	6	3.96	2.25	0.04	4.01	2.28	0.05
N x G	18	77.19	14.62	0.00	77.19	14.62	0.00
R x N x G	18	7.93	1.50	0.09	7.93	1.50	0.09
Error	332	97.36			97.36		
Total	387	903.38			902.70		

R^2=0.89, C.V.=27.97 R^2=0.88, C.V.=28.34

+ All effects except error were assumed to be fixed.

The results of M. incognita race 1 confirmed those of Pasley (1981). Pasley (1981) did find some galling in a resistant ecotype (U.F. No. 55), and attributed this to possible seed contamination. From the results obtained in the present study, it may be that the nematode population used by Pasley (1981) was contaminated with M. incognita race 3.

The gall and egg mass scores were phenotypically correlated with R = 0.7 ($p = 0.05$, MSE = 0.15). As the two scores represent two measures of the same nematodes they were expected to be positively correlated. They were not independent but served different purposes: the gall score was a measure of infection of the plant as well as the survival of the nematode; the egg mass score served as a measure of the nematode reproduction and its response to the environment (in this case the host).

The objective of this experiment was to select genotypes which were different from each other but consistent in their response. In order to assess the consistency of a genotype's response, within a nematode, the heterogeneity of their variance was tested using Bartlett's Test (Snedecor and Cochran, 1967). The mean and variance of each genotype for each nematode species is given in tables 4-14 and 4-15. The corrected Chi Squares, for the genotypes in each nematode species, were close to zero with d.f. = 6, and hence the variances were not

significantly different. The genotypes all responded consistently to an individual nematode species but some genotypes varied among nematode species.

Genotype 189 showed some variation between replications, especially in response to M. incognita race 3. This genotype also varied among nematodes, from fairly resistant to M. arenaria and M. incognita race 1 to intermediate to M. incognita race 3 and M. javanica. This was not an extreme nor consistent genotype and hence was not selected for future work.

There were two clearly susceptible genotypes, 232 and 239. Though 232 varied somewhat in response to M. arenaria it always remained clearly susceptible and tended to be extreme. The most extreme and consistently susceptible genotype was 239.

The intermediate genotypes were 175 and 177. They were not used as parents for further crossing because they had intermediate scores of two and three in most cases, and were not considered always distinguishable.

Only one genotype (167) showed consistently resistant responses to all the nematode species and it was selected as the resistant parent. Genotype 55 also was used as a parent since it usually was resistant or intermediate in response, and as the seed lot originally came from the currently available ecotype that has other desirable traits. However, as 55 showed signs of susceptibility to

Table 4-14. Means and Variances (s^2) of Gall and Egg Mass Scores of Seven Aeschynomene americana Lines Over Two Replications for M. arenaria and M. javanica.

Genotype		Nematode species							
		Meloidogyne arenaria				Meloidogyne javanica			
		Scores				Scores			
		Gall		Egg Mass		Gall		Egg Mass	
	N	Mean	s^2	Mean	s^2	Mean	s^2	Mean	s^2
55	14	0.42	0.28	0.42	0.28	2.95	0.07	2.07	1.78
167	14	0.35	0.64	0.21	0.07	1.57	0.00	1.07	0.07
175	14	2.14	0.28	2.00	0.00	2.87	0.28	1.92	0.07
177	14	2.50	0.07	2.14	0.28	2.64	0.07	1.71	0.28
189	12	0.58	0.08	0.58	0.08	2.23	0.04	1.92	0.08
232	14	3.50	0.64	3.28	1.14	4.92	0.07	4.50	0.07
239	14	4.00	0.00	3.50	0.07	5.00	0.00	4.42	0.00

Table 4-15. Means and Variances (s^2) of Gall and Egg Mass Scores of Seven Aeschynomene americana Lines Over Two Replications for M. incognita race 1 and 3.

Genotype		Nematode species							
		M. incognita race 3				M. incognita race 1			
		Scores				Scores			
		Gall		Egg Mass		Gall		Egg Mass	
	N	Mean	s^2	Mean	s^2	Mean	s^2	Mean	s^2
55	14	1.35	0.28	1.07	0.07	0.92	0.07	0.57	0.00
167	14	0.92	0.07	0.42	0.00	0.42	0.28	0.21	0.07
175	14	2.21	0.64	1.57	2.57	1.28	0.00	0.78	0.64
177	14	3.00	0.00	2.00	0.00	0.85	0.28	0.28	0.28
189	13	1.92	9.23	1.30	3.07	0.28	0.28	0.21	0.64
232	14	3.42	0.00	2.92	0.07	4.42	0.28	4.28	0.28
239	14	4.21	0.07	3.92	0.07	4.85	0.28	4.35	0.07

M. incognita race 3 and M. javanica it was not considered a consistent parent.

In genetic studies it is usual to select the parents at random, so as to be able to apply the results to the entire population. However, in self pollinating species the selection of parents at random causes a conflict. An individual line of a self pollinating species is usually considered homozygous, and therefore does not vary as a heterozygous (open pollinated) species would. There is no genetic variation within a homozygous line, and fewer different genotypes over all. This is because the genes are in a homozygous state, which reduces the number of different genotypes among the lines. For example, a trait controlled by 3 genes can produce a 1:3:3:3:9:9:9:27 phenotypic segregation, but only 8 of the total of 25 different genotypes are homozygous. This also assumes the best possible case where each genotype is distinguishable; more often different homozygous genotypes are similar phenotypically (i.e. where there is dominance). Thus the probability of selecting the same genotype from an entire population, when selecting at random, will be high (10% for a trait controlled by 3 genes). The probability of selecting two genotypes at random which are similar phenotypically is higher still (60% for a trait controlled by 3 genes and some degree of dominance). Genetic effects are measured by the differences or segregations that are

found between genotypes. This can not be achieved if the genotypes are identical or phenotypically indistinguishable. Hence, in self pollinating species, selecting genotypes at random may even make the determination of genetic effects impossible. In order to overcome this conflict, in practice most genetic studies are based on the extremes which represent the widest differences genetically and phenotypically. This then prevents the extrapolation of the results to the entire population. Most genetic studies try to use many parents on both extremes in the hope that it makes a more representative sample, but it is, in fact still not equivalent to random selection.

When a trait is studied which is measured by a scoring system from zero to five, differences among genotypes and phenotypes that are small may not be distinguishable due to the coarseness of the scale. The differences among genotypes may also be the results of environmental effects which the scoring system may not distinguish from genetic effects. This results in fewer parents from which to select for each class. Therefore it is wiser to select parents representing the widest extremes to be able to distinguish genetic and environmental differences. It is also not possible to select large numbers of parents of different genotypes as there are fewer to select from. This limitation may be overcome by using more replications

or numbers of individuals in measuring and testing differences. For future tests and parental selections using this scoring system, it is recommended to test more than seven plants in two replications for each genotype.

Four parental genotypes were selected : 167, resistant; 55, intermediate; 232 and 239, both susceptible. These four represented the largest range in score values (Ø to 5) and in the case of 55 they represent the genotype most common geographically. These parents were also the most consistent in rank and score, among replicates and nematode species.

CHAPTER V
ARTIFICIAL HYBRIDIZATION OF Aeschynomene americana L.

Introduction

Aeschynomene (Aeschynomene americana L.) was recognized as a potential forage legume in the 1950s, and further work showed relatively high nutritional quality, palatability and good growth. A major program of plant introduction and genetic improvement was initiated at the University of Florida in the late 1970s (Kretschmer and Bullock, 1980; Quesenberry and Ocumpaugh, 1981). A collection of over 200 plant introductions and local accessions has been evaluated at both Fort Pierce and Gainesville, Florida for genetic and environmental variation (Kretschmer and Bullock, 1980; Quesenberry and Ocumpaugh, 1981). Much variation was found in numerous agronomic traits including: growth habit (prostrate to erect); hairiness; dry matter production; total crude protein yield; and flowering date, as well as response to pests and diseases.

This species is considered an unimproved species, as it has not yet undergone genetic manipulation in order to become fully domesticated, and combine favorable traits into one genotype. In order to combine the most favorable traits into one genotype, the easiest and most common method used in plant breeding is artificial hybridization.

Aeschynomene is self pollinating, unusual in temperate forage legumes, and little if any cross pollination occurs, especially in the greenhouse; thus it provides the possibility of specific controlled mating. Artificial hybridization will make it possible to create specific new genotypes possibly of superior agronomic value and to study the inheritance of some of the desired traits.

The technique should take advantage of the flowering sequence in order to maximize the success and efficiency of genotype manipulation. The objectives of this research were (1) to understand and determine the flowering sequence in the greenhouse, (2) to develop a technique to create hybrids of aeschynomene, and (3) to define the precautionary measures necessary to insure a reasonable success rate in controlled crosses with minimum chances for cross contamination or self pollination.

Flowering Sequence

Aeschynomene americana is normally a self pollinating species with anthesis occurring after the flower has expanded. We noted that the stigma is surrounded by the stamens in the fused keel, which would maximize the possibilities of self pollination and fertilization. Insects, bees and ants in particular, have been found on the plants, but usually after flower expansion and anthesis, and are thought to be attracted by the excretions of the whole plant rather than the flowers specifically.

Flowering is dependent, to different degrees, on day length, with a reported range in genotype flowering response from approximately 12.5 hours to less than 11 hours (Kretschmer and Bullock, 1980; Quesenberry and Ocumpaugh, 1981). In these studies, a bench with a moveable blackout curtain was used when day length was more than 11 hours. Flowering usually began 30 to 40 days after planting, depending on the genotype, and the health of the individual plant. The flowers are found on axillary racemes, which usually branch from the main stems and are few flowered (Rudd, 1968). Flowering and mature fruit production was prolific on mature plants. Removal of developing flowers and pods caused A. americana to continue flowering, in our case, for nearly a year, thus ensuring a continuous supply of flowers for hybridization.

On any given day, one or rarely two flowers of a raceme reached anthesis. Intervals between successive flowers on one inflorescence varied from one to three days, depending on the climatic conditions. From 12 to 14 hours before anthesis, the bud (2-3 mm long) was completely covered by the bracteoles. During the evening after a sunny day, the corolla and style began to elongate, and continued throughout the night until about 0500 hours when the flower was about 10 mm long.

The stamens started elongating sometime after midnight, usually during the predawn hours, and reached a length of five mm by 0600 to 0800 hours. Pollen was dehisced after

the stamens had elongated between 0500 and 0900 hours but only retained significant viability that day. By nightfall, the pollen was usually whitish and its viability was greatly reduced.

The stigma was receptive from about 0400 to 0500 hours and remained so until about 1600 hours. In a previous preliminary experiment, we found that flowers emasculated at 0400 hours could be pollinated by different pollen sources anytime between 0800 and 1600 hours of the same day, and that there was no difference between genotypes used as females. On warm sunny days, the flowers remained open one day and started to wither towards nightfall. After a cloudy day, flowering was greatly reduced. After self fertilization, the ovary elongated to become a mature pod, the whole process taking about three weeks (Figure 5-1.). Spontaneous abortion was rare except in times of extreme environmental stress such as insect infestation or drought stress.

Artificial Hybridization

When attempting artificial hybridization, usually only one flower bud per raceme was emasculated on the female parent. Occasionally, when two buds were at the appropriate stage of development on one raceme, both were emasculated, but often only one of the two would be successful. The buds at the appropriate stage were between three and five mm long, the bracteoles all but covered the

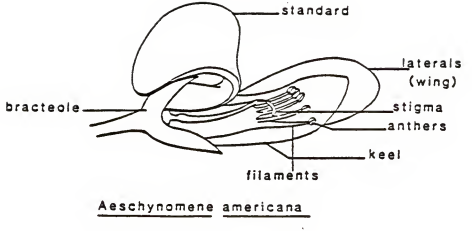

Figure 5-1. Aeschynomene americana Half Flower (x6).

calyx and elongation of the style had started. Usually, 2x or 3x overhead magnifiers were employed when emasculating, because of the very small size of the flower buds. All other buds, flowers, and pods on the inflorescence to be emasculated were removed to prevent any confusion later and to help ensure successful development of the single hybridized flower.

The female flowers could be emasculated between 1800 and 0400 hours. Although success was achieved when emasculating between 0100 and 0400 hours, this time proved to be too inconvenient for extensive and accurate crossing. Generally, the hours between 2100 and 2400 hours were the most advantageous for emasculation though the flowers were smaller at this time than after midnight. An added advantage of pre-midnight emasculation is that the chances of premature stamen development and resulting self pollination are lower at this time.

The flower to be emasculated was held at its base between the thumb and index finger of one hand. The lower bracteole was bent under the bud with a pair of fine forceps and held back with the thumb. The point of a fine pointed forceps was used to split the keel, revealing the two groups of stamens flanking the style. With practice one needed only open the keel slightly and not split it entirely. This resulted in less possible damage to the flower.

Using the forceps, each group of stamens was separated from the style and removed in two groups of five, due to their diadelphic nature. The flower bud was then "repacked" and the bracteole returned to its original position.

The emasculated flowers could be identified in a number of ways but a tag was attached along the internode above the inflorescence with the appropriate information, such as the date and the parentage. The bud appeared normal and opened normally the following morning; the only evidence of interference being an occasional split keel.

Pollination may take place between 0800 and 1600 hours but preferably in the morning. The pollen was collected from the male parents on flat toothpicks to which a small piece of fine emery paper had been attached. The pollen was rubbed onto the stigmatic surface, the capitate apex of which held the pollen. Between each pollination combination, the toothpicks and hands were washed in 70% ethanol to prevent contamination. The tag was then marked with the appropriate parentage information.

The humidity in the greenhouse was usually sufficiently high for rapid pollen germination. Attempts were made to increase the humidity around individual flowers by bagging the flower in glassine bags with wet cotton. However, the flowers were often disturbed to such an extent that they dropped off during or after pollination. It is therefore suggested that the best way to ensure rapid pollen

germination is to maintain a high humidity in the entire greenhouse rather than around an individual flower and risk damaging it.

Pollen remained viable for 12 hours when collected in the morning, kept dry and stored under refrigeration. No success was achieved in pollinating the females at night immediately after emasculating them. This was probably due to the possibility that the style was not yet receptive at 0900 to 1200 hours. When wetted, the pollen formed clumps and could not be used; therefore, it is not recommended to water the plants before pollinating the flowers.

The success of a cross was determined in three to five days by the visible elongation of the ovary and in two weeks by the development of the pod. If the cross was not successful, the flower or pod would abort and abscise anytime between three and five days from pollination.

The pods were harvested about three to four weeks after crossing, depending on climatic conditions. Usually, between one and seven seeds (mean of four in our studies) were produced per cross, regardless of the genotype. There was a large amount of variation in the number of seeds per pod and, therefore, the crossing success was expressed in terms of number of pods produced per crossed flower. Overall, the success percentage ranged between 40 and 60 pods per 100 pollinations but could be as high as 80 for some individuals carrying out crossing on certain days.

The success of hybridization was independent of the female parent used (Table 5-1), and of the male parent as well (Table 5-2). The success rate should and did improve with practice and was certainly dependent on the individual carrying out the process, as well as the environmental conditions.

Precautions

The precautions below will minimize the risks of self pollination and maximize the success of the cross.

(1) Remove new flowers developing on the inflorescence after a flower has been crossed, for at least two weeks, to ensure the development of the cross product and to eliminate confusion at harvest.

(2) Avoid damage of the petal while emasculating. If the keel is damaged during emasculation, it may be removed and the cross may still be successful but the success rate is reduced.

(3) Avoid bending the style much during emasculation as minute cracks can be observed in the style which cause the flower to abscise in two or three days.

(4) Remove the stamens entirely rather than picking off the anthers individually. This procedure causes minimum damage to the style and is the fastest method of emasculation. When the anthers are removed individually, the chance of selfing would increase due to anthers or pollen left behind inadvertently. A self pollinated flower

Table 5-1. Effect of Eleven Female Parents on Pod and Seed Production of Artificially Hybridized Aeschynomene americana L.

Female parent ID	Different males	Total flowers emasculated	Total pods set	Total seeds set	Pods per flower emasculated
	----------	number	----------		%
55	5	154	91	346	59.09ns
167	5	161	97	371	60.24
170	2	28	15	63	53.57
176	2	33	17	93	51.50
189	3	41	18	84	43.90
195	3	19	10	34	52.63
197	4	60	25	86	41.60
221	5	52	22	96	42.30
232	4	103	53	210	51.46
239	5	135	79	309	58.51
241	2	20	9	36	45.00

ns = percentages not significantly different for this 11 x 2 Contingency Table: Chi square = 9.18, d.f. = 10, P = 0.75-0.5.

Table 5-2. Effect of Eight Male Parents on Pod and Seed Production of Artificially Hybridized Aeschynomene americana L.

Male parent ID	Different females	Total flowers emasculated	Total pods set	Total seeds set	Pods per flower emasculated
	----------number----------				%
55	9	166	99	349	59.63ns
167	6	114	63	270	55.26
169	3	30	14	50	46.60
178	3	26	13	43	50.00
189	6	127	49	195	38.50
232	8	150	82	353	54.66
239	3	148	87	355	58.78
240	3	46	20	96	43.47

ns = percentages not significantly different for this 8 x 2 Contingency table: Chi square = 7.85, d.f. = 7, P = 0.5-0.25.

usually develops with visible staminal remains (filaments) which are persistent up to the time of harvest. When the stamens are removed entirely, there are no visible remains and the pod is recognizably different from a self pollinated one.

(5) Refold the flower after emasculation to reduce the chances of foreign pollen contamination, and help ensure a normal continued development.

(6) Maintain as constant as possible greenhouse temperature and relative humidity. High temperatures and low humidities increase the spontaneous abortion and abscission of selfed and artificially hybridized flowers.

(7) Identify each cross with the initials of the individual making the cross, the date and parentage information in order to have a means of measuring the confidence in the cross.

Plant Maintenance and Seed Collection

Aeschynomene americana is an annual herb, and thus short lived. If properly timed, the plants can be maintained almost indefinitely through cuttings. A section of the plant, including a few nodes, is excised and either planted directly in soil or left in water for a week or less and then planted. The cutting will produce roots in about three days in water or take root in about a week if planted in soil. If the mother plant is under severe environmental stress (i.e. drought or nutrient) or

otherwise unhealthy (i.e. disease or insect infestation) the cuttings are unlikely to take or survive much longer than a week at maximum. Flowering can be maintained by often removing the flowers on an individual plant. Flowering will eventually cease when the plant becomes too old.

Seeds produced by artificial hybridization or selfing, take about three weeks to mature. If harvested too early, the seeds are of reduced viability and germinate poorly. Seeds were always collected from each flower individually, if from crosses or selfed generations. A small paper coin envelope was used to store the seed, with the appropriate information marked on the outside. The seeds were generally stored under refrigeration, which tended to improve their viability at germination and reduce the occurrence of seed born diseases, and may well have served as a vernalization treatment.

Aeschynomene is a hard seeded legume and therefore needs to be scarified when germinated (Ruelke et al. 1975; Hanna, 1973). In all the experiments carried out, the seeds were scarified by either scratching the seed coat with fine emery paper or soaking the seeds in sulfuric acid (full strength) for ten minutes and rinsing thoroughly with distilled water. Once scarified, the seeds were left to germinate in petri dishes with filter paper and distilled water. Germination usually took about two to three days at $26^{o}C$ ($80^{o}F$).

CHAPTER VI
EFFECTS OF *Aeschynomene americana* ON THE LIFE CYCLE OF A ROOT-KNOT NEMATODE (*Meloidogyne*) SPECIES

Introduction

The mechanisms involved in nematode resistance in most plant species are not well understood and can vary among plant and nematode species. Resistance can be defined as the collection of properties of the host which do not favor the parasite. This may be translated into how large or small the aptitude a host offers to the development of the species considered (Bingefors, 1982). The resistant host may submit to the parasite aggression but not enable it to multiply normally. For agricultural purposes, host resistance is demonstrated by the prevention of the nematode feeding, developing, and reproducing on the plant which would cause economic losses (Bingefors, 1982). Resistance may also be anything that prevents, retards or restricts a disease (Veech, 1981).

Nematologists refer to two forms of resistance (Giebel, 1982) (1) Passive or pre-infectional and (2) Active or post-infectional. Passive resistance is conditioned by anatomical, physiological or chemical barriers which hinder the invasion of the nematode or disrupts its ontogenesis.

This form of resistance may not be related to nematode resistance per se, as it is always present in the host regardless of nematode attack and is part of the host's normal physiology or anatomy. Active or post-infectional resistance is induced by the nematode. An endogenous or constitutive substance is synthesized specifically for the eradication of the nematode or protection of the host from the nematode. In any case, resistance in one way or another results in a mechanism affecting some part of the nematode life cycle in connection with its parasitic mode of action. Taylor and Sasser (1978) list five possible effects of resistance on the nematodes:

1. juveniles develop into mature females but cannot reproduce.
2. juveniles develop into males.
3. juveniles never mature to an adult.
4. the nematode at any stage is killed by the immune reaction.
5. the nematode leaves the root.

In conjunction with an investigation of the inheritance of "resistance" to root-knot nematodes, the effect of Aeschynomene americana genotypes on the life cycle of Meloidogyne incognita race 3 was studied. The objectives were to determine (1) the parts in the life cycle of M. incognita race 3 which are affected by resistant or tolerant genotyes of A. americana, and (2) if the

intermediate response resulted from fewer mechanisms functioning or reduced efficiency of the mechanism(s) that were effective on a more resistant genotype.

Materials and Methods

Three Aeschynomene americana L. plant introduction lines were selected, based on their responses to Meloidogyne incognita race 3 in previous experiments, as determined by gall and egg mass scores described in Chapter III. Florida No. 167 was resistant with a gall and egg mass score ranging from 0 to 1. Florida No. 55 was considered intermediate with a gall and egg mass score ranging from 0 to 2. Florida No. 239 was susceptible with a gall and egg mass score ranging from 3 to 5.

The seeds were scarified, germinated and grown in 150 cm^3 Conetainers (Ray Leach Conetainer Nursery, Canby OR.), filled with methyl bromide fumigated Arredondo fine sand topsoil (loamy, silicious, hyperthermic, Grossarenic Paleudult). The seedlings were grown in the greenhouse, inoculated with cowpea type rhizobium, and watered daily.

The M. incognita race 3 was grown on "Rutgers" tomatoes in a growth room for at least 60 days. The eggs were collected using 1.05% sodium hypochlorite and by sieving through 200-mesh sieve (0.149-0.074 mm openings) and a 500-mesh (0.028 mm openings) sieve. The eggs or juveniles were maintained in an aqueous suspension by continuous

stirring, and injected into the soil around the seedling via a re-pipetting syringe and left in the soil for the duration of the experiment.

Experiment 1.

The plants were grown in the greenhouse from 17 Sept. through 19 Nov. 1984, under short day lengths (i.e. <12 hours) and with air temperature ranging from 21 - 32 ^{o}C. Two weeks after germination, each plant was individually infested with 300 nematode eggs in an aqueous suspension. The plants were first sampled 28 days after infestation and at seven day intervals for four weeks thereafter. At each harvest, 14 plants from each genotype were uprooted, washed free of soil and examined. From each genotype seven of the plants were stained with Phloxine B (see staining procedure) in order to count the number of egg masses. The egg masses were then removed from the females and placed in a solution of 0.5% sodium hypochlorite (NaOCl), which released the eggs from the gelatinous matrix. The number of eggs found on a root system were counted in a graduated 'Cooper Tissue Culture Dish' (60 x 15 mm) and the number per egg mass determined. The remaining seven plants were stained with acid fuchsin (see staining procedure) to stain the nematodes in situ. The number of egg masses, eggs per egg mass, various juvenile stages, and adult females were recorded for each plant root system.

Experiment 2.

The plants were grown from 10 April through 28 June, 1985, under long days (>12 hours) and with air temperatures ranging from 21 to 40 ^{o}C. Two weeks after germination, they were individually infested with 200 freshly hatched second stage juveniles ("J2") in an aqueous solution. Four plants of each genotype were harvested 40 hours after inoculation and weekly for seven weeks thereafter. Egg masses were counted without staining. The number of eggs per egg mass and the individual nematodes in situ were counted as in Experiment 1.

Staining Procedures

Phloxine B.

Roots were washed free of soil, then immersed in an aqueous solution of Phloxine B stain (15 mg L^{-1}) for 15 minutes. This stained the gelatinous matrix of the egg mass bright red, making egg masses readily visible to the unaided eye for the assessment of the egg mass scores.

Acid Fuchsin.

This staining method, slightly modified from that of Byrd et al. (1983), facilitated the observation of the nematodes within the root tissues. The roots were cleared in diluted (1.0 %) NaOCl very briefly (30 to 60 secs.), then rinsed very thoroughly with water and left in water for 15 minutes. Care was taken at this point to avoid overexposure of the roots to the bleach, as they could

disintegrate if the solution is too strong or they are left in it too long (especially when the roots were young and still very fine).

Roots were then transferred to beakers with dilute acid fuchsin stain, (1 ml of stain stock solution to 30 ml of water; stain stock solution = 3.5 g acid fuchsin + 250 ml acetic acid + 750 ml distilled H_2O). They were then boiled in the stain for 30 secs. and cooled to room temperature. After rinsing off the excess stain, the roots were placed in glycerin (acidified with a few drops of 5N HCl) or acetic acid. Though the original procedure of Byrd et al. suggested boiling the roots again while in the glycerin, it proved too detrimental in this case, disintegrating the roots beyond the point of usefulness. The stained roots were observed in petri dishes or between microscope slides using either a dissecting or compound microscope.

Though it would seem feasible, the two staining techniques could not be successfully combined. Once Phloxine B was applied to a root, it prevented adequate staining of the same tissue with acid fuchsin. Each staining method was adequate for its own purpose and simple to use, but they were incompatible.

It was difficult to distinguish the juvenile stages in situ. The second juvenile stage ("J2") is the longest in duration and most variable in length. In this study, the category "J2" included all juveniles found in the root

tissues that retained the slender filiform body shape; thus it included those freshly hatched from egg masses that developed in the root and those that had recently penetrated the root in search of infection sites. The "J3/J4s" included all juveniles whose bodies had increased in diameter and had obviously lost mobility and probably included some advanced J2.

Results and Discussion

From both experiments it can be seen that infection took place over several days (See tables 6-1 and 6-2). The J2's are the invading stage, and responsible for penetration. Griffin and Elgin (1977) found a progressive increase in the rate of penetration of M. hapla into both resistant and susceptible varieties of alfalfa, even seven days after inoculation. Our data showed a similar trend, but reduced J2 penetration was not distinguishable from J2's leaving the root system in the more resistant genotypes.

The life cycle of the nematode always took longer in the more resistant genotypes. Egg production, for example, did not occur until after 42 days (Table 6-1) or 38 days (Table 6-2), whereas in the susceptible genotype eggs were found after 35 days (Table 6-1) and 23 days (Table 6-2). It may have been that nematode development was accelerated in the susceptible genotype, suggested by the large production of eggs after 35 days in experiment 1 and the

Table 6-1. Nematode Life Cycle Stages Found in Three Aeschynomene americana Genotypes under Short Days and Cool Temperatures, when Infested with M. incognita race 3 (Experiment 1).

	Nematode Life Cycle Stages														
	J2			Juveniles			Females			Egg masses /plant			Egg /Egg mass		
Time from infestation	167	55	239+	167	55	239	167	55	239	167	55	239	167	55	239
Days	total number++									number					
28	Ø	Ø	19	Ø	54	123	5	1Ø	59	Ø	Ø	Ø	Ø	Ø	Ø
35	Ø	6	1Ø	Ø	37	61	Ø	6	32	Ø	1	2	Ø	31Ø	456
42	Ø	Ø	Ø	Ø	Ø	4	Ø	25	57	1	1	3	164	468	485
5Ø	Ø	Ø	Ø	Ø	7	Ø	Ø	6	36	1	1	3	622	687	678

\+ U.F. Plant Introduction genotypes.
++ total number found in seven plants

Table 6-2. Nematode Life Cycle Stages Found in Three Aeschynomene americana Genotypes under Long Days and Warm Temperatures, when Infested with M. incognita race 3 (Experiment 2).

Time from infestation	Nematode Life Cycle Stages														
	J2			Juveniles			Females			Egg masses /plant			Egg /Egg mass		
	167	55	239+	167	55	239	167	55	239	167	55	239	167	55	239
Days	---------- total number ++ ----------									---------- number ----------					
1.7	19	21	38	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	2	6	30	1	1	30	0	0	0	0	0	0
16	0	0	1	2	6	35	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	3	36	70	0	4	19	0	69	68
31	0	0	0	0	0	0	0	18	110	0	1	59	0	112	211
38	0	0	13	0	0	1	11	18	226	9	12	94	141	145	256
46	0	0	0	0	0	0	4	15	260+	0	3	233	0	118	132
54	0	10	25	0	7	32	0	21	450	0	14	268	0	163	159

\+ U.F. Plant Introduction Genotypes.
++ total number found in four plants

turn-over in generations after 38 days in experiment 2. Minton (1962) found that the life cycle of M. incognita var acrita took longer in more resistant cotton species. Moreover, he noted that in the most resistant species, the nematode did not advance past the "J2" and "J3" stage and no nematodes were found in these species after 121 days.

The life cycle took longer and fewer nematodes matured to adults in Experiment 1 (Table 6-1) than in Experiment 2 (Table 6-2) in all the genotypes. This suggested that the life cycle of the nematodes was affected by the reduced day length and change in temperature. Thus it appeared that a relationship existed between the mechanism for resistance and the physiological effects of day length and temperature on the host. With cooler temperatures and shorter days the life cycle was prolonged, as it was in the resistant genotype under long days and warmer temperature.

Minton (1962) suggested that the failure of the cotton root cells to respond to the nematodes resulted in fewer nematodes developing to maturity and less gall formation. He also suggested that resistance was not due to root barriers, but to a restraint on larval development, as resistant genotypes showed a high level of initial infection but greatly reduced larval development and gall formation. The same type of restraint on nematode development was observed in our experiments, where development seemed delayed and fewer nematodes survived in the more resistant genotypes. In the resistant genotype,

some nematodes could reach maturity and reproduce normally in Experiment 1 (with the longer days and cooler temperatures). For example, 50 days after inoculation in Experiment 1, the susceptible, intermediate and resistant genotypes had 3, 1, and 1 egg masses per plant which yielded 678, 687 and 622 eggs/egg mass respectively. Whereas, under long days and warmer temperatures (Experiment 2) the number of eggs/egg mass was reduced in the resistant and intermediate genotypes compared with the susceptible. In Experiment 2, 38 days after inoculation, the susceptible, intermediate and resistant genotypes had 94, 12 and 9 egg masses per plant which yielded 265, 145 and 141 eggs/egg mass, respectively. The results from both experiments, suggests a strong effect of the environment on the fecundity of the nematodes, which may have overridden the resistance mechanism in certain environments (i.e. under shorter day lengths and cooler temperatures).

Thus under the quite different growing conditions of these experiments, progressively stronger resistance was characterized by:

1. progressively slower maturation and egg mass production.

2. a reduction of the numbers of individuals that eventually matured and produced egg masses.

Males were found in both experiments, associated with egg masses formed on the roots of all genotypes but predominantly in the more resistant genotypes. As the

procedures used could not assure finding all the males, only their presence was noted. The occurrence of males in resistant genotypes suggested that perhaps the resistance mechanism caused an environmental stress for the developing nematodes, thus stimulated development of males via sexual reversal (Papadopoulou and Triantophyllou, 1982).

Overall, the main effect of resistance was to prevent the juveniles developing into mature, reproducing adults. The resistance appeared to work by retarding maturation past the juvenile stages, which may have caused the actual reduction in nematode numbers. Under shorter days and cooler temperatures, resistance seemed to have no effect on reproduction (i.e. eggs produced). This suggested that perhaps resistance affected maturation and the environmental conditions (i.e. those factors other than the host genotype) affected fecundity. The environmental factors functioned only in the more adverse conditions; under optimal conditions for the nematode the host's resistance mechanism(s) could function. If there were several mechanisms functioning or reduced efficiency in certain genotypes they could not be determined due to the strong interaction with the environmental effects. The intermediate response appeared to be a form of resistance but was tempered by the environmental effects on the plant genotype and the nematodes.

CHAPTER VII
INHERITANCE OF ROOT-KNOT NEMATODE RESISTANCE IN *Aeschynomene americana* L.

Introduction

The genetic system controlling a trait in self pollinating species may be determined by intercrossing homozygous lines and examining the responses in the succeeding generations. The responses of F1's indicate whether the trait is dominant, recessive or additively controlled. This is because the F1's are in theory 100% heterozygous and identical and vary only due to environmental effects. Deviations from one or both parents indicate the type of gene action.

The selfing of an F1 creates the F2 generation, which in a self pollinating species is the generation where there is the maximum amount of segregation possible. From this segregation, genetic ratios and models can be determined when the environmental effects are uniform or minimized. If the F1's are back crossed to their respective parents, the model for the F2 may be confirmed by the resulting segregation.

The objective of this investigation was to determine the genetic system controlling the response to three

root-knot nematode species in aeschynomene. This was to be achieved by examining the responses of the parents, F1's, F2's and several backcrosses to M. incognita race 3, M. arenaria race 1, and M. javanica.

Materials and Methods

Generations

From the parent data (Chapter IV), four parents were selected to represent the widest range in responses to the nematode species, and the least variation within a line. Three plants were used of each genotype and these were crossed in all combinations by the method described in Chapter V, to create F1 hybrids (Table 7-1). The parents were coded such that 167 = 1; 55 = 2; 239 = 3; and 232 = 4. Their respective F1's and F2's were coded in the same manner, with the female parent numbered first (i.e. 1x2 = 167 as female crossed with 55 as the male). One of each F1 was then grown to maturity and F2 seed was collected. Unfortunately not all the F1's grown out survived and some extra plants had to be used (See Table 7-1). A few backcrosses were attempted, but due to insect problems in the greenhouse many of the F1 plants used for these did not survive long enough to make many backcrosses or collect the seeds of all the reciprocal F2's.

Table 7-1. Identification of Parents and F1's[+] Used for the Evaluation of the Inheritance of Resistance to Root-knot Nematodes.

Female parent	Male Parent			
	1 (167)	2 (55)	3 (239)	4 (232)
1 (167)	1	1x2 (2)	1x3 (1)	1x4 (1)
2 (55)	2x1 (2)	2	2x3 (1)	2x4 (3)
3 (239)	3x1 (3)	3x2 (1)	3	3x4 (1)
4 (232)	4x1 (2)	4x2 (1)	4x3 (1)	4
BC female parent				
1x2	x 1	x 2		
1x3	x 1	-	x 3	
2x4	-	x 2	-	
4x3	-	-	x 3	x 4

+ The number of F1's used for the backcrosses and the F2's are in parentheses after each F1.

Evaluation

In April, 1985, the crosses and generations were tested for their responses to each of the following nematode species, Meloidogyne incognita race 3, M. arenaria race 1, and M. javanica. For each nematode species or treatment, seven plants were grown of each of the four parent lines (28 total), seven plants for each of the twelve F1's (84 total), 16 of each backcross (112 total), 126 plants of each F2 (1134 total), (see Appendix A), totalling 1358 plants per nematode treatment, and 4074 plants in all.

The plants were grown in Conetainers in the greenhouse and infested with their respective nematode treatments in the manner described in Chapter IV. The nematode eggs were obtained in the manner described in Chapter III and were from the populations maintained by F. E. Woods in the Nematology laboratory. It was believed that the M. incognita race 3 population used in this study was the same as that which was derived from the North Carolina Host Differential Test in Chapter III.

The plants were individually scored for gall and egg mass score in the manner described in Chapter IV, after eight weeks of infestation.

Results

The gall scores were more useful than the egg mass scores, as they showed greater differences between responses. This is because the gall scores were always

higher than egg mass scores within each cross. Therefore, only the gall score data were used for the interpretation of the data. Nevertheless, staining the egg masses made the galls more visible and thus facilitated the scoring.

The intermediate scores found in the parent population as a whole (Chapter VI) and in the F1 and F2 generations suggests that the trait may be quantitative. If nematode resistance were controlled by four genes for example, then there would be less than one F2 plant in the homozygous 0 class from a resistant by susceptible cross (0.39%). In all the resistant by susceptible (and vice versa) crosses there were more than 0.39% of the F2's found in the 0 class (see appendix A for actual numbers). Thus, though the trait at first appears to be quantitative, the fact that so many F2's were in the 0 class suggested that the trait was in fact controlled by a few major genes.

To view the responses as a qualitative trait presented a difficulty of where to make the cutoff point for resistance. The 0-1 and 4-5 classes were obviously categorized as resistant and susceptible, respectively. The difficulty lies in the distinction between the 2 and 3 classes, both of which seemed to indicate levels of tolerance rather than resistance. Resistance, defined in agricultural terms, is that reaction which prevents the nematode from feeding, developing, and reproducing on the plant to a level which would cause economic losses

(Bingefors, 1982). With this definition in mind, the 2 class was usually classed as resistant or intermediate and the 3 class as susceptible even though at the low end it could be classed as tolerant. The data were separated into two categories: Ø to 2 as resistant; and 3 to 5 as susceptible (Tables 7-5, 7-6, and 7-7).

The means for M. javanica and M. arenaria were unexpectedly low for the susceptible parents and the susceptible by susceptible crosses (Parents 3 and 4 on Table 7-2 and 7-3). The means for the parents inoculated with M. incognita were similar to those found in Chapter IV (Table 7-4). The mean gall scores of the F1 and F2 generations from all crosses were noticeably more resistant than their parents (except for the resistant by resistant cross) (Table 7-4).

Most of the resistant by susceptible crosses and their reciprocals gave resistant F1's (See Means in Table 7-2, 7-3, and 7-4). The F1 means from these crosses were somewhat higher than the those obtained for the resistant parents, which suggested partial dominance or additive gene action.

From samples collected from other experiments in 1985 and 1986, it was determined by the Division of Plant Industries (DPI) of the Florida Dept. of Agriculture and Consumer Services and the Nematology laboratory of I.F.A.S. that the nematode populations used may have been

Table 7-2. Mean Gall and Egg Mass Score for All Generations and Families Inoculated with *M. javanica*.

Generation	N	Gall	Egg	Generation	N	Gall	Egg
Parents				Parents			
1(167)	7	0.5	0.00	3(232)	7	2.8	2.0
2(55)	7	0.2	0.14	4(239)	7	3.0	1.6
F1				F1			
1x2	7	2.0	0.57	2x1	7	2.0	1.42
1x3	7	2.2	2.28	3x1	7	2.0	1.42
1x4	7	0.1	0.00	4x1	7	0.7	0.14
2x3	7	1.2	0.42	3x2	7	1.1	1.14
2x4	7	0.0	0.00	4x2	7	0.7	0.57
3x4	7	3.1	2.42	4x3	7	1.1	0.85
BC				BC			
(1x2)x1	16	3.18	1.37	(1x2)x2	16	2.68	1.51
(1x3)x1	16	2.30	1.56	(1x3)x3	16	1.3	0.43
(2x4)x2	16	1.12	1.5				
(4x3)x3	16	1.18	0.75	(4x3)x4	16	1.18	0.93
F2				F2			
1x2	126	2.4	1.61	2x1	-	-	-
1x3	127	1.8	1.38	3x1	-	-	-
1x4	126	1.3	0.82	4x1	124	1.46	0.6
2x3	126	2.7	2.16	3x2	-	-	-
2x4	126	1.7	1.12	4x2	126	0.92	0.49
3x4	125	1.6	1.22	4x3	125	1.42	0.54

Table 7-3. Mean Gall and Egg mass Score for All Generations and Families Inoculated with *M. arenaria* race 1.

Generation	N	Gall	Egg	Generation	N	Gall	Egg
Parent				Parent			
1(167)	7	0.0	0.0	3(232)	7	1.28	1.57
2(55)	7	0.0	0.14	4(239)	7	1.00	3.6
F1				F1			
1x2	7	0.0	0.57	2x1	7	0.0	0.42
1x3	7	1.8	0.42	3x1	7	1.6	2.14
1x4	7	0.1	1.28	4x1	7	0.0	1.42
2x3	7	0.3	1.14	3x2	7	1.6	2.85
2x4	7	0.3	2.00	4x2	7	0.4	3.71
3x4	7	2.57	4.42	4x3	7	1.8	4.42
BC				BC			
(1x2)x1	16	0.25	1.06	(1x2)x2	16	0.06	0.81
(1x3)x1	16	0.75	0.68	(1x3)x3	16	1.38	2.31
(2x4)x2	16	0.87	0.81				
(4x3)x3	16	1.25	2.93	(4x3)x4	16	0.93	3.87
F2				F2			
1x2	126	0.32	0.77	2x1	-	-	-
1x3	126	1.13	1.68	3x1	-	-	-
1x4	126	0.75	1.47	4x1	126	0.80	2.40
2x3	127	1.93	2.44	3x2	-	-	-
2x4	126	0.50	1.85	4x2	126	0.50	1.86
3x4	126	1.07	2.68	4x3	126	2.15	3.64

Table 7-4. Mean Gall and Egg mass Score for All Generations of all Families Inoculated with *M. incognita* race 3.

Generation	N	Gall	Egg	Generation	N	Gall	Egg
Parents				Parents			
1(167	7	0.0	0.0	3(232)	7	4.5	1.6
2(55)	7	1.0	0.14	4(239)	7	3.1	3.6
F1				F1			
1x2	7	0.7	0.57	2x1	7	0.7	0.42
1x3	7	0.8	0.42	3x1	7	2.4	2.14
1x4	7	1.7	1.28	4x1	7	2.0	1.42
2x3	7	1.2	1.14	3x2	7	3.0	2.85
2x4	7	2.0	2.00	4x2	7	3.0	3.71
3x4	7	5.0	4.42	4x3	7	4.4	4.42
BC				BC			
(1x2)x1	16	1.18	1.06	(1x2)x2	16	1.25	0.81
(1x3)x1	16	1.18	0.68	(1x3)x3	16	3.31	2.31
(2x4)x2	16	1.37	0.81				
(4x3)x3	16	3.00	2.93	(4x3)x4	16	3.93	3.87
F2				F2			
1x2	126	1.03	0.77	2x1	-	-	-
1x3	127	1.82	1.68	3x1	-	-	-
1x4	126	1.64	1.47	4x1	124	2.56	2.40
2x3	126	2.70	2.44	3x2	-	-	-
2x4	126	2.00	1.85	4x2	126	2.04	1.86
3x4	125	2.90	2.68	4x3	125	3.70	3.64

Table 7-5. F2 Segregation Ratios for All Crosses Inoculated with M. javanica

Cross	Gall Score 0-1	2	3	4-5	N	Chi^2
1x2						
Observed	-	73	53	-	126	
Expected (1:1)		63	63			3.16
Expected (9:7)		70.8	55.2			0.15
3x4						
Observed	54	38	33	-	125	
Expected (3:1)		93.75	31.25			0.13
4x3						
Observed	70	47	8	1	126	
Expected (15:1)		118.125	7.87			0.17
1x3						
Observed	50	29	44	4	127	
Expected (3:1)		95.25	31.75			11.08
2x3						
Observed	2	22	99	4	126	
Expected (3:13)		23.6	102.4			0.01
1x4/4x1						
Observed	141	55	51	2	250	
Expected (3:1)		187.5	62.5			1.82
2x4/4x2						
Observed	137	64	50	1	252	
Expected (13:3)		204.75	47.25			0.37
Expected (3:1)		189	63			3.04

Table 7-6. F2 Segregation Ratios for All Crosses Inoculated with *M. arenaria* race 1.

Cross	Gall Score 0-1	2	3	4-5	N	Chi^2
1x2						
Observed	126	-	-	-	126	
Expected (1:0)	100		0			0.00
4x3						
Observed	13	75	36	2	126	
Expected (0:1)		100				1.34
3x4						
Observed	78	26	21	1	126	
Expected (3:1)		94.5	31.5			3.82
1x3						
Observed	80	23	20		126	
Expected (3:1)		94.5	31.5			3.05
2x3						
Observed	40	35	51	1	127	
Expected (1:3)	31.7	95.3				2.85
1x4						
Observed	98	14	14	-	126	
Expected (3:1)	94.5	31.5				0.51
4x1						
Observed	95	15	14	2	126	
Expected (3:1)	94.5	31.5				0.01
2x4/4x2						
Observed	164	56	19	13	252	
Expected (13:3)		204.75	47.25			6.05
Expected (10:5)	157.5	94.5				0.72

Table 7-7. F2 Segregation Ratios for All Crosses Inoculated with M. incognita race 3.

Cross	Gall Score				N	Chi^2
	Ø-1	2	3	4-5		
1x2						
Observed	91	35	-	-	126	
Expected (1:Ø)		1ØØ	Ø			Ø.ØØ
3x4/4x3						
Observed	4	2Ø	13Ø	97	252	
Expected (Ø:1)		Ø	1ØØ			2.48
1x3						
Observed	54	33	26	13	126	
Expected (3:1)		94.5	31.5			2.38
2x3						
Observed	6	38	69	13	126	
Expected (1:3)		31.5	94.5			6.61
1x4						
Observed	56	42	22	6	126	
Expected (3:1)		94.5	31.5			Ø.52
4x1						
Observed	14	45	49	18	126	
Expected (7:9)		55.12	70.87			Ø.48
2x4/4x2						
Observed	77	96	53	25	251	
Expected (3:1)		188.25	62.75			1.23

contaminated with other root-knot nematode species. However, the exact nature of the contamination in terms of species and proportions can not been determined. This possible contamination may account for the loss of susceptibility in the susceptible by susceptible cross (3x4/4x3) in the F1 and F2 generations, in M. javanica especially. It also seems likely that this contamination distorted the segregations of the F2 and backcross generations, for example 2x4/4x2 and 2x3 (See Table 7-6 and 7-7 respectively).

The data for each nematode species were classified into the two categories mentioned earlier and their segregation ratios were tested by Chi^2 Tests with 1 degree of freedom (Tables 7-5, 7-6, and 7-7). Where there was no difference in the means for a pair of reciprocal crosses, the F2 data were combined.

Infesting the plants with M. javanica gave anomalous results in the F2's (Table 7-5). The resistant by resistant cross (1x2) fit a 9:7 ratio nearly perfectly but there was no resistance score lower than a 2. The susceptible by susceptible crosses (3x4 and 4x3) gave moderately susceptible F1's but almost all the F2 means were resistant (Table 7-2). The ratios that most closely explained the F2 segregations, in the resistant by susceptible crosses, were dominant for resistance (Table 7-5). A possible genetic explanation of this may be

linkage or complimentary gene action of multiple alleles. The resistant by susceptible crosses and their reciprocals all fit simple gene models of one or two genes (i.e. 1x4/4x1 3:1 or 13:3; 2x4/4x2 13:3 or 3:1) except for 1x3 and 2x3 (Table 7-5). No genetic model was found which could explain the segregations. It would seem possible that the susceptible genotypes, 3 and 4, have different genes or combinations of alleles which were competing with each other. The intermediate genotype, 2, seemed dominant over the resistant genotype (1). Resistance from genotype 1, was dominant to the susceptible types (3 and 4), and the intermediate resistance from 2 was dominant over 4 but not over 3.

The means for all the crosses and generations inoculated with M. arenaria were inexplicably low, especially for the susceptible parents 3 and 4. For this reason the segregation ratios (Table 7-6) were calculated using 1 as the cutoff point. This cutoff point gave satisfactory ratios for 1x2, 4x3, 1x3, 1x4, and 4x1 which indicated the same or similar sorts of genetic relationships as with M. javanica. In this case the 2x4/4x2 crosses were unexplainable, there being too many resistant F2's. Unfortunately, the backcross data did not give any satisfactory segregation ratios and could not be explained.

The segregation ratios for M. incognita were similar to those found in the other nematode populations and had acceptable Chi^2 tests for most of the crosses. There were two exceptions: 2x3 and 4x1, which both had too many plants in the middle range of 2 - 3. Perhaps it is in situations such as this that the scoring system needs more divisions to more precisely classify reactions.

As the means for the different nematode treatments were not very different and the nematode treatments may have been very similar (combinations of species), the F2 segregations were combined (Table 7-8).

In the combined data (Table 7-8), the resistant by intermediate parents (1x2) did not fit a Chi^2 Test for 1:0 but also did not show a high level of susceptibility (the 53 susceptible all came from M. javanica). Therefore, they were regarded as possibly genetically identical or nearly identical if there was a close linkage between two genes.

The more surprising data were those of the susceptible by susceptible crosses (3x4/4x3) which at a cutoff score of 1, had numerous resistant F2's. Most of the resistant types from these crosses also came from the M. javanica data, but some also came from 3x4 in M. arenaria. This could be explained by a close linkage which when forced to recombine gave rise to resistant genotypes. Otherwise, these peculiar data may be due to the inoculum used.

Table 7-8. F2 Segregation Ratios for All Crosses for All Nematode Species.

Gall Class	N	Cross Observed	Expected	Ratio	Chi^2
		1x2			
0-2	378	325	378	1	
3-5		53	0	0	7.43
		1x4/4x1			
0-2	754	575	565.5	3	
3-5		178	188.5	1	0.743
		1x3			
0-2	379	269	284.25	3	
3-5		110	94.75	1	3.350
		2x3			
0-2	379	143	142.12	6	
3-5		236	236.87	10	0.007
		2x4/4x2			
0-2	755	594	613.43	13	
3-5		161	141.56	3	3.280
		3x4/4x3			
0-1	755	219	188.75	1	
2-5		536	566.25	3	6.464
0-1			235.93	5	
2-5			519.06	11	1.767

For those resistant by susceptible crosses using 1 as the resistant parent, the F2's segregated 3:1. This would suggest a single dominant gene was involved. The 2x4/4x2 crosses gave a 13:3 ratio for resistance, suggesting that 4 was the double recessive. Only the 2x3 cross really did not fit any satisfactory ratio, apart from a 6:10 (an additive ratio) which would indicate that parent 2 (55) was a double recessive. This suggested that either the contamination was such that the resistance did not function completely or that 167 and 55 have different genes. The resistant genotype 167, would then have been homozygous for the dominant allele which confers resistance and 2 (55) would have been homozygous for the recessive alleles which conferred only intermediate resistance. If 2 (55) had more than one gene segregating in the F2 and 1 (167) had only one, then one might expect them to segregate more when they were crossed together (1x2).

Discussion

Root-knot nematode resistance in A. americana appeared to be controlled by relatively few genes. The simple genetic ratios identified account for most of the data and crosses. No other model fit as well. No combination of genes or genetic model was found which could account for all the crosses.

The range in galling seemed to indicate a quantitative trait but there were too many plants in the supposedly

homozygous class of 0. Therefore it was supposed that only a small number of genes were involved. The F1 means from resistant by susceptible crosses tended towards the resistant parent but not completely. This indicated that a partial dominance or combination of dominance and additive gene action was involved.

Approximately half of each F2 from these crosses were found in the 2 and 3 categories, which suggested a natural division of resistance and susceptibility between these classes. This division may function as a threshold for nematode development, such that once within either the `susceptible' or `resistant' classes, the actual extent of the nematode development was environmentally controlled (i.e. all factors other than the host genotype).

Such a division into a binary code between the 2 and 3 class demonstrates the greatest difficulty that arose during this investigation. That was obtaining a precise gall classification of individual plants. While there were some heavily galled and obviously susceptible plants and others which were free or nearly free of galls and obviously resistant, the majority of plants were found in the intermediate classes.

The egg mass scores showed less than half the variation that the gall scores gave and hence were not used in obtaining categories or ratios. The staining of the egg mass did, however, emphasize the galls and facilitated the scoring process.

After this investigation had taken place, it was found that the root-knot nematode populations used were probably contaminated with other root-knot nematode species. The exact nature of the contamination in the populations used in these experiments in terms of species and proportions can not be determined. The results of the supposedly different nematode inoculation treatments did not appear to differ greatly. The possible contamination seemed to distort the F2 segregations of certain crosses more than others, notably 2x3, 2x4/4x2, and the susceptible by susceptible 3x4/4x3. This may explain why no one genetic model or system could be found to explain all the data.

In theory, all F1's from a pair of homozygous parents are identical. In this case, the parents were assumed to be homozygous as they were selected for the uniformity of their responses and due to their method of reproduction. But there were no marker genes or other means of positively identifying F1 hybrids from selfs. The particular F1 plant used to make backcrosses and F2's was not the plant(s) used to evaluate the response, though in theory all F1's are identical. Certain F1's were used to create F2's and backcrosses and others were used for testing the responses due to the nature of the effect of the nematode on a host plant. Even mildly affected plants do not produce great numbers of seeds. As the F2's all segregated more than the F1's, it is likely that they came from true hybrids.

Though this element of doubt in the identity of the F1's exists, there was no evidence of selfing from other experiments, (not reported here and using the same method of artificially hybridizing described in Chapter V). The assumption used in this analysis and interpretation of the data was that the F1's were all identical and the F2's came from true hybrids. The assumption could be proven by incorporating a marker gene to test for hybridization, or developing a means of using F2's from F1's with known responses (i.e. by using cuttings).

The cross 1x4 (167x232) and 4x1 (232x167) gave a consistent 3:1 ratio in the F2's for all three nematode species individually and combined. This suggested that there might be a gene controlling the response which was common to all three nematode species and that it was dominant. Unfortunately, 232 was considered somewhat variable as a parent and no backcrosses were made with it, which would confirm the hypothesis of one dominant gene.

In conclusion, there appears to be at least one genetic system which is common to all three nematode species. But there may be another level of control which may be a pair of linked genes working additively or in partial dominance which is specific to the individual nematode species.

In order to resolve this question further work would be necessary. In particular, this should include testing more F1's and test crosses to determine if there is linkage, and examining the segregations from known F1's and F2's.

APPENDIX

Table A-1. Data Summary from 1986 Experiment of Aeschynomene americana parents, F1's, F2's and Backcrosses which were Inoculated with Meloidogyne incognita race 3.

Generation	N	Gall Score						Egg mass Score					
		0	1	2	3	4	5	0	1	2	3	4	5
parents													
1 (167)	7	7	-	-	-	-	-	7	-	-	-	-	-
2 (55)	7	-	7	-	-	-	-	4	3	-	-	-	-
3 (239)	7	-	-	-	-	3	4	-	-	-	-	3	4
4 (232)	7	-	1	-	3	3	-	-	1	-	3	3	-
F1s													
1x2	7	2	5	-	-	-	-	3	4	-	-	-	-
1x3	7	1	6	-	-	-	-	4	3	-	-	-	-
1x4	7	-	2	5	-	-	-	1	3	3	-	-	-
2x1	7	2	5	-	-	-	-	4	3	-	-	-	-
2x3	7	2	1	4	-	-	-	3	-	4	-	-	-
2x4	7	-	-	7	-	-	-	-	-	7	-	-	-
3x1	7	-	-	4	3	-	-	-	-	6	1	-	-
3x2	7	-	-	-	7	-	-	-	-	4	3	-	-
3x4	7	-	-	-	-	-	7	-	-	-	2	-	5
4x1	7	-	-	7	-	-	-	-	4	3	-	-	-
4x2	7	-	-	-	7	-	-	-	-	-	2	5	-
4x3	7	-	-	-	2	-	5	-	-	-	2	-	5
BCs													
(1x2)x1	16	5	5	5	-	1	-	6	5	4	-	1	-
(1x2)x2	16	2	8	6	-	-	-	6	7	3	-	-	-
(1x3)x1	16	5	3	8	-	-	-	8	5	3	-	-	-
(1x3)x3	16	1	1	7	6	-	4	1	1	8	5	-	1
(2x4)x2	16	5	1	9	1	-	-	8	4	3	1	-	-
(4x3)x4	16	-	-	-	3	11	2	-	-	1	2	11	2
(4x3)x3	16	-	1	-	13	2	-	-	1	1	12	2	-
F2s													
1x2	126	30	61	35	-	-	-	49	56	21	-	-	-
1x3	126	21	33	33	26	12	1	26	30	40	19	10	1
1x4	126	24	32	42	22	5	1	29	36	40	15	5	1
2x3	126	-	6	38	69	12	1	3	15	45	50	12	1
2x4	126	8	25	55	33	5	-	14	26	55	26	5	-
3x4	126	1	3	18	84	17	2	4	7	35	61	17	2
4x1	126	5	9	45	49	13	5	9	13	47	37	15	5
4x2	125	11	33	41	20	20	-	21	35	29	20	20	-
4x3	126	-	-	2	46	62	16	-	2	9	37	62	16

Table A-2. Data Summary from 1986 Experiment of Aeschynomene americana parents, F1's, F2's and Backcrosses which were Inoculated with Meloidogyne javanica.

Generation	N	Gall Score						Egg mass Score					
		Ø	1	2	3	4	5	Ø	1	2	3	4	5
Parents													
1 (167)	7	3	4	-	-	-	-	7	-	-	-	-	-
2 (55)	7	5	2	-	-	-	-	6	1	-	-	-	-
3 (239)	7	-	-	1	6	-	-	-	1	5	1	-	-
4 (232)	7	-	-	-	7	-	-	-	4	2	1	-	-
F1s													
1x2	7	-	-	7	-	-	-	3	4	-	-	-	-
1x3	7	-	-	1	6	-	-	-	1	3	3	-	-
1x4	7	6	1	-	-	-	-	7	-	-	-	-	-
2x1	7	-	-	7	-	-	-	-	4	3	-	-	-
2x3	7	2	1	4	-	-	-	4	3	-	-	-	-
2x4	7	7	-	-	-	-	-	7	-	-	-	-	-
3x1	7	-	-	7	-	-	-	-	4	3	-	-	-
3x2	7	2	3	1	1	-	-	2	3	1	1	-	-
3x4	7	-	-	-	6	1	-	-	1	3	2	1	-
4x1	7	2	5	-	-	-	-	6	1	-	-	-	-
4x2	7	2	5	-	-	-	-	3	4	-	-	-	-
4x3	7	4	-	1	2	-	-	5	-	-	2	-	-
BCs													
(1x2)x1	16	-	-	1	11	4	-	3	5	7	1	-	-
(1x2)x2	16	-	-	5	11	-	-	1	7	7	1	-	-
(1x3)x1	16	-	2	7	7	-	-	3	4	6	3	-	-
(1x3)x3	16	3	9	3	1	-	-	12	2	1	1	-	-
(2x4)x2	16	2	11	2	1	-	-	2	5	8	1	-	-
(4x3)x4	16	4	6	5	1	-	-	7	4	4	1	-	-
(4x3)x3	16	5	6	3	2	-	-	10	2	2	2	-	-
F2s													
1x2	126	-	-	73	53	-	-	5	47	66	8	-	-
1x3	127	23	27	29	44	3	1	45	23	29	26	3	1
1x4	126	43	35	15	31	1	1	76	13	21	15	1	-
2x3	126	1	1	22	98	4	-	7	25	38	52	4	-
2x4	126	14	36	39	37	-	-	40	38	40	8	-	-
3x4	125	20	34	38	33	-	-	45	29	29	22	-	-
4x1	124	22	42	40	20	-	-	81	19	16	7	1	-
4x2	126	38	49	25	13	1	-	84	24	17	-	1	-
4x3	126	13	57	47	8	1	-	82	24	16	3	1	-

Table A-3. Data Summary from 1986 Experiment of Aeschynomene americana parents, F1's, F2's and Backcrosses which were Inoculated with Meloidogyne arenaria race 1.

Generation	N	Gall Score						Egg mass score					
		Ø	1	2	3	4	5	Ø	1	2	3	4	5
parents													
1 (167)	7	7	-	-	-	-	-	7	-	-	-	-	-
2 (55)	7	7	-	-	-	-	-	7	-	-	-	-	-
3 (239)	7	3	1	1	2	-	-	4	1	1	1	-	-
4 (232)	7	4	-	2	1	-	-	4	-	2	1	-	-
F1s													
1x2	7	7	-	-	-	-	-	7	-	-	-	-	-
1x3	7	-	1	6	-	-	-	1	6	-	-	-	-
1x4	7	6	1	-	-	-	-	7	-	-	-	-	-
2x1	7	7	-	-	-	-	-	7	-	-	-	-	-
2x3	7	5	2	-	-	-	-	7	-	-	-	-	-
2x4	7	6	-	1	-	-	-	7	-	-	-	-	-
3x1	7	-	3	4	-	-	-	-	3	4	-	-	-
3x2	7	1	1	5	-	-	-	1	1	5	-	-	-
3x4	7	1	-	-	6	-	-	2	-	3	2	-	-
4x1	7	7	-	-	-	-	-	7	-	-	-	-	-
4x2	7	4	3	-	-	-	-	5	2	-	-	-	-
4x3	7	2	-	2	3	-	-	2	-	3	2	-	-
BCs													
(1x2)x1	16	13	2	1	-	-	-	14	1	1	-	-	-
(1x2)x2	16	15	1	-	-	-	-	15	1	-	-	-	-
(1x3)x1	16	11	-	3	2	-	-	11	1	4	-	-	-
(1x3)x3	16	6	1	6	3	-	-	6	1	6	3	-	-
(2x4)x2	16	8	3	4	1	-	-	9	3	3	1	-	-
(4x3)x4	16	9	1	4	2	-	-	10	-	5	1	-	-
(4x3)x3	16	8	2	-	6	-	-	9	1	-	6	-	-
F2s													
1x2	126	85	41	-	-	-	-	102	24	-	-	-	-
1x3	126	55	25	23	20	3	-	61	35	14	13	3	-
1x4	126	73	25	14	14	-	-	81	27	14	4	-	-
2x3	127	21	19	35	51	1	-	25	33	32	36	1	-
2x4	126	82	30	9	5	-	-	92	23	7	4	-	-
3x4	126	61	17	26	21	1	-	79	11	24	11	1	-
4x1	126	75	20	15	14	1	1	78	26	11	10	-	1
4x2	126	82	26	10	8	-	-	95	27	2	2	-	-
4x3	126	7	6	75	36	2	-	28	22	49	25	2	-

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BIOGRAPHICAL SKETCH

Stephanie Randa Langley Hardy was born on the 25th of April 1960 in Beirut, Lebanon , of British parents.

Stephanie attended the United Nations School of New York from 1967 to 1972. In 1973, Stephanie attended the European School of Brussels, Belgium. She graduated with a European Baccalaureate in 1978 (with specialties in English, French, mathematics and physics). During 1979, Stephanie was employed by the engineering department of the Brussels Hilton as an executive secretary.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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